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BIOLOGICAL BULLETIN

EXPERIMENTS WITH CHRYSOMELID BEETLES.

III. The Effects of Killing Parts of the Eggs of *Leptinotarsa decemlineata*.[1]

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1. Introduction.

The method used in the experiments described in this paper, *i. e.*, killing parts of the egg, has been employed by a number of investigators in many ways and for many purposes. Heat and electricity are the agents which have been most frequently applied. So far as I know insects' eggs have heretofore never been operated upon in this way. The results obtained by the use of these agents are quite similar to those so frequently brought about by removing parts of the egg or embryo, or by isolating blastomeres. The latter process is of course impossible in the case of the insect's egg, since cleavage is superficial, but material has been removed successfully from different parts of beetles' eggs in various stages of development.[2]

Three years ago a preliminary report was made of experiments in removing portions of the eggs of *Calligrapha multipunctata*,

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 131. Parts I. and II. of "Experiments with Chrysomelid Beetles" appeared in the Biological Bulletin, Vol. XIX., June, 1910, pp. 18–30.

C. bigsbyana, *C. lunata* and *Leptinotarsa decemlineata*.[1] Before the results of those experiments and the data contained in the present contribution can be understood clearly, a brief account of the structure of freshly laid chrysomelid eggs and of the principal stages in their normal embryonic development is necessary.

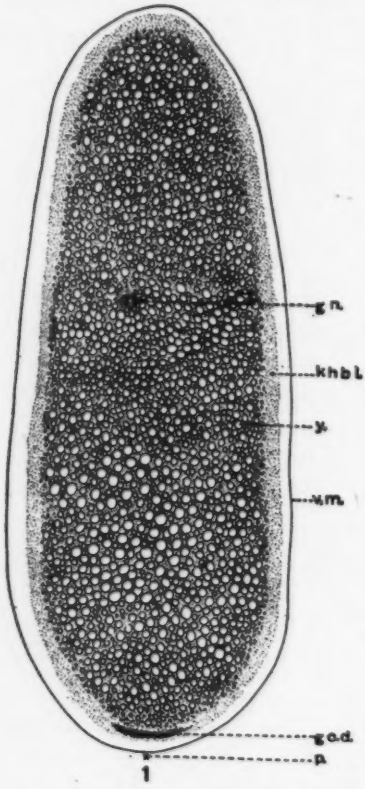

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FIG. 1. A longitu inal section through an egg of *Calligrapha bigsbyana* four hours after deposition. *gcd*, germ cell determinants; *gn*, germ nuclei copulating; *khbl*, "Keimhautblastem"; *p*, posterior; *vm*, vitelline membrane; *y*, yolk. The eggs of *Leptinotarsa decemlineata* are not visibly different from those of *C. bigsbyana*.

The freshly laid egg (Fig. 1) consists of a large central mass of yolk globules (*y*) and a comparatively thin superficial layer of cytoplasm, the "Keimhautblastem" (*khbl*). Two envelopes cover the egg, the vitelline membrane (*vm*) and the chorion. Polar bodies are usually present at this time, and the egg nucleus is in the act of union with the sperm nucleus (*gn*), or else cleavage has already begun. Embedded in the "Keimhautblastem" at the posterior end of the egg is a disc-shaped mass of darkly staining granules, which I have called the pole-disc, or germ cell determinants (*gcd*).

As cleavage progresses, a separation of the cleavage products into sections occurs; the

[1] Hegner, R. W., '08*b*, "The Effects of Removing the Germ Cell Determinants from the Eggs of Some Chrysomelid Beetles," BIOL. BULL., Vol. 16

nuclei of one group form a more or less regular layer equidistant from the periphery, and later migrate outward, fuse with the "Keimhautblastem" and become the blastoderm (Fig. 2, *bl*). The nuclei of the other group remain behind in the yolk (Fig. 2, *v*), which it is their duty to dissolve. Eight of the cleavage products which reach the posterior end do not help to form the blastoderm, but gather the germ cell determinants about them and continue their migration until they are entirely separated from the egg; these are the primordial germ cells (Fig. 2, *pgc*).

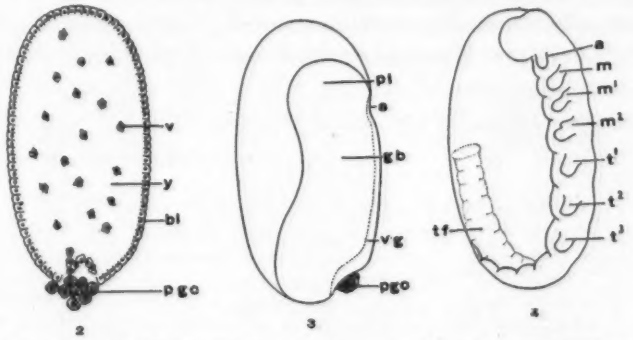

FIG. 2. A longitudinal section through an egg of *Leptinotarsa decemlineata* one day after deposition when in the blastoderm stage. *bl*, blastoderm; *pgc*, primordial germ cells; *v*, vitellophag; *y*, yolk.

FIG. 3. Superficial view of the right side of an egg of *Leptinotarsa decemlineata* thirty-six hours after deposition. *gb*, germ band; *pgc*, primordial germ cells; *pl*, procephalic lobes; *s*, stomodeum; *vg*, ventral groove.

FIG. 4. Surface view of right side of an egg of *Leptinotarsa decemlineata* forty-eight hours after deposition. *a*, antenna; *m*, mandible; *m'*, first maxilla; *m²*, second maxilla; *t'*–*t³*, thoracic appendages; *tf*, tail fold.

They remain quiescent for a considerable period and then migrate into the embryo, which has developed in the meantime.

At the end of two days the germ band appears on the ventral surface of the egg (Fig. 3, *gb*); this lengthens within the next twenty-four hours, growing forward to the extreme anterior end, and posteriorly until the tail-fold reaches over half way up on the dorsal surface (Fig. 4, *tf*). During this elongation the embryo segments and the appendages of the head and thorax grow out. The entire embryo then contracts antero-posteriorly and begins to grow around the yolk (Fig. 5). This contraction continues

until the end of the tail-fold coincides with the posterior end of the egg (Fig. 6). The larva (Fig. 7) usually appears in five or six days.[1]

2. Killing the Germ Cell Determinants.

The method used in the experiments mentioned above to remove the germ cell determinants was to prick the posterior end of the freshly laid egg with a needle and allow them to flow out. These experiments were not considered entirely successful, since I was unable to determine in any case whether all of the germ cell determinants had been removed. Some of the embryos which developed from eggs operated upon in this way produced

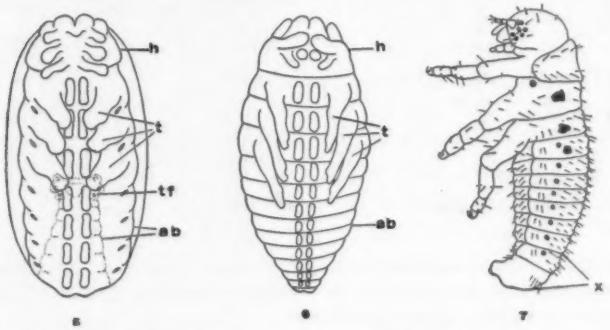

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Fig. 5. Ventral view of an egg of *Leptinotarsa decemlineata* sixty hours after deposition. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

Fig. 6. Ventral view of an egg of *Leptinotarsa decemlineata* seventy-two hours after deposition. *ab*, abdomen; *h*, head; *t*, thoracic appendages.

Fig. 7. Side view of a newly hatched larva of *Leptinotarsa decemlineata*.

either a lesser number of germ cells than normal, or else no germ cells at all. The conclusion was reached that if all of the germ cell determinants are removed from the egg no germ cells will be produced, and that the granules which constitute the pole disc are really germ cell determinants.[2]

Two series of experiments (L.D. 09 and L.D. 018) were carried

[1] Hegner, R. W., '08*a*, "Observations on the Breeding Habits of Three Chrysomelid Beetles, *Calligrapha bigsbyana*, *C. multipunctata* and *C. lunata*, *Psyche* Vol. 15.

[2] For a discussion of this subject see Hegner, R. W., '11, "The Germ Cell Determinants in the Eggs of Chrysomelid Beetles," *Science*, Vol. XXXIII., pp. 71–72.

out in order to learn whether or not germ cells would appear in embryos if the germ cell determinants were prevented from taking part in their development. Certain data regarding these experiments are listed in Tables I. and II. Eggs were oriented as soon as laid and the central region of the posterior end of each was touched with a hot needle, thus killing the protoplasm just beneath in which the germ cell determinants were embedded. Since it was impossible to see the germ cell determinants in the living egg, a rather large area had to be killed in order to be certain that all of them had been reached. As noted above, the experiments in removing the germ cell determinants by pricking the egg and allowing them to flow out were not entirely successful because it was never possible to tell whether all of them had been obtained.

TABLE I.

EXPERIMENTS IN KILLING THE GERM CELL DETERMINANTS.

Leptinotarsa decemlineata—Series L.D. 09.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 09 A	Control.			Hatched in 6 days.
L.D. 09 B1	Immediately after deposition (see Fig. 1).	Posterior end killed with hot needle.	0	4 cleavage nuclei present.
L.D. 09 B2			1 day.	See Fig. 8.
L.D. 09 B3			2 days.	
L.D. 09 B4			3 days.	See Fig. 11.
L.D. 09 B5			4 days.	
L.D. 09 B6			5 days.	
L.D. 09 B7			7 days.	

TABLE II.

EXPERIMENTS IN KILLING THE GERM CELL DETERMINANTS.

Leptinotarsa decemlineata—Series L.D. 018.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 018 A	Control			Hatched normally.
L.D. 018 B1	Immediately after deposition (see Fig. 1).	Posterior end killed with hot needle.	0	2 cleavage nuclei found.
L.D. 018 B2			1 day.	
L.D. 018 B3			2½ days.	See Fig. 9.
L.D. 018 B4			3 days.	
L.D. 018 B5			4 days.	
L.D. 018 B6			6 days.	

Many of the eggs operated upon in experiments L.D. 09 and L.D. 018 did not develop at all or developed abnormally. Under

ordinary circumstances eggs sometimes become shapeless masses of tissue or remain in the condition of the freshly laid egg, but this fact does not explain the great number of cases observed among the operated eggs. It is evident that many of the operated eggs failed to develop because of the conditions of the experiments. For this reason only a few of the best defined embryos have been selected for descriptive purposes.

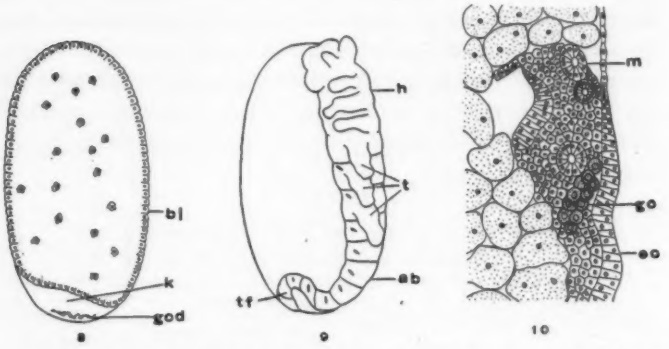

FIG. 8. Longitudinal secion through an egg of *Leptinotarsa decemlineata* (L.D. 09 B2). The posterior end was killed with a hot needle just after the egg was laid (see Fig. 1); the egg was then allowed to develop for twenty-four hours. *bl*, blastoderm; *gcd*, germ cell determinants; *k*, portion of egg killed.

FIG. 9. Side view of an egg of *Leptinotarsa decemlineata* (L.D. 018 B3). The posterior end was killed with a hot needle just after deposition (see Fig. 1); the egg was then allowed to develop for sixty hours. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

FIG. 10. Longitudinal section through the tail fold of a normal embryo of *Leptinotarsa decemlineata* sixty hours old, showing the germ cells (*gc*). *ec*, ectoderm; *m*, malpighian tubules.

Fig. 8 is from a longitudinal section of an egg (L.D. 09 B2) which was operated upon just after deposition and was then allowed to develop for twenty-four hours. This should be compared with the normal egg at a similar stage of development (Fig. 2). In the egg shown in Fig. 8 the germ cell determinants (*gcd*) and a portion of the neighboring yolk and cytoplasm (*k*) have been prevented from taking part in development. That part of the egg which remained alive produced a blastoderm of a single layer of cells (*bl*). The principal difference to be noted

between this preparation and that of a normal embryo is the absence of the primordial germ cells at the posterior end (see Fig. 2, *pgc*). Three perfect series of sections were cut from eggs at this stage, but none disclosed any germ cells.

Fig. 9 is a sketch of embryo L.D. 018 B3 (see Table II.). It was killed two and one half days after the operation and is a stage a trifle younger than that shown in Fig. 5. The tail fold (*tf*), however, is not fully developed as in Fig. 5. Longitudinal sections were cut through two embryos like that shown in Fig. 9, but no germ cells could be found. Fig. 10 was drawn from a section through the tail fold of a normal embryo two and one half days old; it indicates where the germ cells (*gc*) are situated at this time. If germ cells had been present in the embryo shown in Fig. 9, they would certainly have been found.

These experiments demonstrate that germ cells are not produced when the extreme posterior end of the egg is prevented from taking part in development, and it seems probable from the method of origin of the germ cells that the destruction of the germ cell determinants is the real cause of their absence.[1]

3. Killing the Primordial Germ Cells.

Table III. gives the data for the experiments performed in series L.D. 011. Sixty-nine eggs were laid at 11 A.M. June 24,

TABLE III.

EXPERIMENTS IN KILLING THE PRIMORDIAL GERM CELLS.

Leptinotarsa decemlineata—Series L.D. 011.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 011 A	Control.			Hatched normally.
L.D. 011 B1	One day after deposition (see Fig. 2).	Posterior end killed with hot needle.	0	Like Fig. 2.
L.D. 011 B2			1 day.	
L.D. 011 B3			2 days.	
L.D. 011 B4			3 days.	
L.D. 011 B5			4 days.	
L.D. 011 B6			5 days.	One hatched.
L.D. 011 B7			6 days.	Several hatching.

[1] The writer's papers on "The Origin and Early History of the Germ Cells in some Chrysomelid Beetles" (*Journ. Morph.*, Vol. 20) and on "Germ Cell Determinants and Their Significance" now in press (*American Naturalist*) discuss these points fully.

and were allowed to develop until 11 A.M. June 25. The posterior end of sixty-five of them was then killed with a hot needle. The four controls hatched on June 29. The eggs when operated upon were in a stage like that shown in Fig. 2. One of the operated eggs hatched on June 30 (L.D. 011 B6), several were hatching on the following day, and a number of others were ready to hatch at that time. The one that hatched and three of those ready to hatch were examined and then sectioned and stained. Superficially they resembled the larva shown in Fig. 7, the only difference being the absence of the last two posterior segments which are indicated by the letter *x* in Fig. 7; one possessed all but the last segment. The sections showed that none of these larvæ contained germ cells.

It is evident from these experiments that the primordial germ cells were killed by the operation and no new ones were produced by the developing embryos. This is, I believe, the earliest stage at which surgical castration has been performed among the Insecta. The influence of this operation upon secondary sexual characters could not be determined, since none of these characters make their appearance in the larvæ.

4. Killing Parts of Freshly Laid Eggs.

The experiments described under heading 2, "Killing the Germ Cell Determinants," might be included in this part of the paper, but they were considered of sufficient importance to warrant a special account. In performing the experiments in series L.D. 09 (Table I.) it was found impossible to regulate with any degree of exactness the amount of the egg killed, and, since it was absolutely necessary that all of the germ cell determinants be killed, a larger portion of the egg was killed than desired. Some of these eggs, however, when allowed to continue their development, provided data with regard to the effects of killing a large part of the posterior end of freshly laid eggs.

Fig. 11 was drawn from an egg from series L.D. 09 B4. The portion killed by the operation is labelled *k*; the material that remained alive produced the head (*h*) and part of the thorax (*t*) of an embryo. These parts resemble the corresponding parts of a normal embryo at a like age (see Fig. 6). Apparently that

part of the "Keimhautblastem" which remained alive after the operation, became supplied with nuclei, was broken up into cells, and proceeded to develop that particular part of the embryo to which it would have given rise if the rest of the egg had not been killed.

Two other series of experiments, L.D. 07 and L.D. 08, were performed to test these results and in every case the living part of the egg developed as though the entire egg were intact. None of the tissue that is normally produced by the killed portion was regenerated by the living material. The embryos developed up to the time of hatching, but were unable to break out of the chorion.

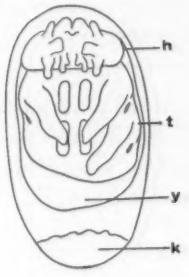

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FIG. 11. Ventral view of an egg of *Leptinotarsa decemlineata* (L.D. 09 B4). The posterior end (*k*) was killed just after deposition (see Fig. 1); a normal head (*h*) and part of the thorax (*t*) developed from the material which remained alive. *y*, yolk.

5. KILLING PARTS OF EGGS IN THE BLASTODERM STAGE.

The eggs used for the experiments designated as series L.D. 04 (Table IV.) were laid at 4 P.M. on June 16, and operated upon at 4 P.M. June 17. They were, at the time of the operation, in a stage similar to that of the egg shown in Fig. 2. As indicated in Table IV., two kinds of operations were performed; the anterior part of one half of the eggs was killed with a hot needle, and the posterior part of the other half was killed in a like manner. Three preparations have been selected to show the results of these experiments, (1) L.D. 04 A2, Fig. 12, (2) L.D. 04 A3, Fig. 13, and (3) L.D. 04 B3, Fig. 14.

The embryos shown in Figs. 12 and 13 developed from eggs which had their posterior parts killed, and were fixed four days and seven days later respectively. One of these embryos (Fig. 12) consists of a head and thorax which appear to be normal in every respect, and are as fully developed as these parts in a normal embryo at a similar age (five days). The abdomen of this

TABLE IV.

EXPERIMENTS IN KILLING PARTS OF EGGS IN THE BLASTODERM STAGE.
Leptinotarsa decemlineata—Series L.D. 04.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 04 A1	Control.			Hatched normally.
L.D. 04 A2	Blastoderm stage (see Fig. 2).	Posterior part killed.	4 days.	See Fig. 12.
L.D. 04 A3			7 days.	See Fig. 13.
L.D. 04 B1		Anterior part killed.	0	
L.D. 04 B2			3 days.	
L.D. 04 B3			4 days.	See Fig. 14.
L.D. 04 B4			7 days.	

embryo is entirely missing. The conclusion is reached that the part of the blastoderm which would have produced the abdomen of the embryo was killed in the operation, and that none of this region was regenerated by the tissue which remained alive.

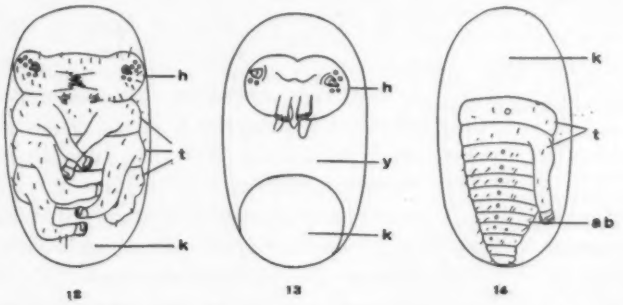


FIG. 12. Ventral view of an egg of *Leptinotarsa decemlineata* five days old (L.D. 04 A2). The posterior end of the egg (*k*) was killed when in the blastoderm stage (see Fig. 2); the blastoderm which remained alive produced a normal head (*h*) and thorax (*t*).

FIG. 13. Ventral view of an egg of *Leptinotarsa decemlineata* eight days old (L.D. 04 A3), operated upon as in Fig. 12. Only a head (*h*) developed from the tissue which remained alive.

FIG. 14. Side view of an egg of *Leptinotarsa decemlineata* five days old (L.D. 04 B3). The anterior end of the egg (*k*) was killed when in the blastoderm stage (see Fig. 2); the blastoderm which remained alive produced a normal abdomen (*ab*) and part of the thorax (*t*).

Fig. 13 was drawn from an egg that was fixed three days later than that of Fig. 12, *i. e.*, at the age of eight days. Only the head of this embryo developed. It is apparent that not only

the tissue destined to produce the abdomen, but also that set aside to form the thorax was killed by the operation. The region of the blastoderm which normally develops into the head covers a considerable area at the time the operation was performed. This area lessens in extent when the germ band arises (Fig. 3, *pl*), and, after the cephalic appendages appear (Fig, 4, *a*, *m*, m^1, m^2), the anterior end of the embryo shortens until the mouth parts are closely crowded together (Figs. 5, 6 and 7). In egg L.D. 04 A3 (Fig. 13) the shortening of the embryo has resulted in the uncovering of a large yolk area (*y*), and, though a comparatively small part of the egg was killed (*k*), this portion bore the blastoderm which in normal eggs gives rise to the larger part of the embryo, *i. e.*, the thorax and abdomen.

When the blastoderm surrounding the anterior end of the egg is killed, only the posterior embryonic region develops from the part which remains alive. This is shown in egg L.D. 04 B3, Fig. 14. Here the normal number of abdominal segments appear as well as two thoracic segments (*t*), one of which has developed a normal pair of legs.

6. Killing Parts of Young Embryos.

The series of figures numbered 15 to 18 show what takes place when parts of young embryos are killed and are thus prevented from continuing development. Table V. gives the data of the operations. The eggs, fifty-two in number, were laid at 10 A.M. June 26; the operations were performed at 10 A.M. June 28, at which time the eggs bore embryos similar to that shown in Fig. 4. Four of the eggs were kept as controls; these hatched on July 2. Approximately one half of the anterior end of twenty-four of the eggs was killed with a hot needle; the posterior half of the other twenty-four eggs was killed in like manner. In every instance the part of the embryo which remained alive developed as though the egg had not been disturbed.

Figs. 15 and 16 show two stages in the development of the posterior part of the embryo, and Figs. 17 and 18 show corresponding stages in the development of the anterior part of the embryo. It is interesting to note that in the egg shown in Fig. 15 not only the anterior end of the embryo, but also the extreme

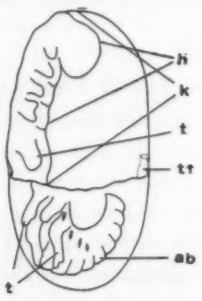
TABLE V.

EXPERIMENTS IN KILLING PARTS OF YOUNG EMBRYOS.

Leptinotarsa decemlineata—Series L.D. 016.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 016 A	Control.			Hatched normally.
L.D. 016 B1	Young embryo (see Fig. 4).	Anterior part killed.	0	See Fig. 4.
L.D. 016 B2			1 day.	See Fig. 15.
L.D. 016 B3			2 days.	
L.D. 016 B4			3 days.	
L.D. 016 B5			4 days.	See Fig. 16.
L.D. 016 C1		Posterior part killed.	1 day.	See Fig. 17.
L.D. 016 C2			2 days.	See Fig. 18.
L.D. 016 C3			3 days.	
L.D. 016 C4			4 days.	

end of the tail fold (*tf*) was killed by the operation, and that the intermediate region consisting of two thoracic segments and eight abdominal segments continued to develop normally except for the mechanical difficulties interposed by the killed material.



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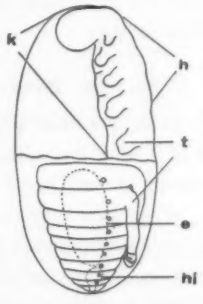

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FIG. 15. Side view of an egg of *Leptinotarsa decemlineata* three days old. (L.D. 016 B2). The anterior end (*k*) was killed when the embryo had reached the stage shown in Fig. 4; the posterior end continued to develop. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

FIG. 16. As in Fig. 15, six days old (L.D. 016 B5). *e*, enteron; *hi*, hind intestine.

An *in toto* preparation of an older embryo from this material (Fig. 16) shows that the living part of the embryo succeeded in growing around the yolk and developing a hind intestine (*hi*) which grew forward toward the yolk-filled enteron (*e*).

The preparation shown in Fig. 17 is from an egg fixed one day after the posterior end had been killed, and is the same age as that of Fig. 15, *i. e.*, three days old. It is of special interest, since the end of the tail fold (*tf*), which was not killed by the operation, has continued to develop, although it is an extremely small piece of tissue and was separated from the rest of the living embryo by a considerable amount of yolk. The anterior part of the embryo consisting of the cephalic region and the first thoracic segment, developed normally. During the twenty-four hours between the operation and fixation, the living part of the

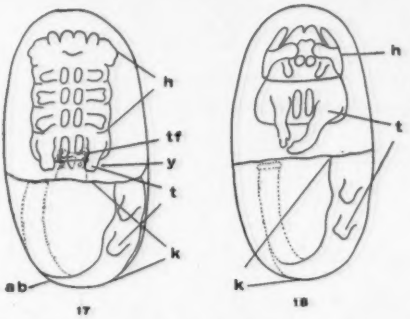

FIG. 17. Side view of an egg of *Leptinotarsa decemlineata* three days old (L.D. 016 C1). The posterior end (*k*) was killed when the embryo had reached the stage shown in Fig. 4; the anterior end continued to develop and has come to lie on the right side of the egg. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold; *y*, *yolk*.

FIG. 18. As in Fig. 17 four days old (L.D. 016 C2).

embryo contracted and left a large yolk space (*y*) between it and the killed material (*k*). A similar condition was noted above in series L.D. 04 A3, Fig. 13 (*y*).

Fig. 18 represents an embryo (L.D. 016 C2) which was allowed to live one day longer than that just described. Here the head and first thoracic segment have continued to develop reaching a stage similar to that shown in Fig. 6. This part of the embryo has changed its orientation since the operation and now lies on the right side of the egg instead of on the ventral surface. Several other cases like this were observed in series L.D. 016 and in a number of the embryos from other series of experiments.

The last egg selected from this series was fixed three days after the operation at an age of five days. It indicates that development of the living part of the embryo proceeds up to the time of hatching.

7. Killing Parts of an Old Embryo.

The eggs used for these experiments were laid at 4 P.M. June 17, and operated upon at 4 P.M. June 20, at the age of three days. Part of them were kept as controls; the rest were divided into two lots and operated upon as indicated in Table VI. The control eggs hatched on June 22.

TABLE VI.

Experiments in Killing Parts of Old Embryos.

Leptinotarsa decemlineata—Series L.D. 06.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 06 A	Control			Hatched normally.
L.D. 06 B1	Old embryo (see Fig. 6).	Posterior end killed.	0	See Fig. 6.
L.D. 06 B2			1 day.	
L.D. 06 B3			2 days.	
L.D. 06 B4			4 days.	
L.D. 06 C1		Anterior end killed.	1 day.	
L.D. 06 C2			2 days.	
L.D. 06 C3			4 days.	

Fig. 6 shows a normal embryo fixed at the time of the operation. The eggs under experimentation were fixed at intervals and stained and mounted. In every case the part of the embryo that remained alive continued to develop. This was true for both those with the anterior part and those with the posterior part killed. There were no signs of regeneration even after the normal embryonic period had passed. The killed part of the embryo began to disintegrate immediately after the operation.

8. Summary.

1. If the region of a freshly laid egg of *Leptinotarsa decemlineata*, which contains the germ cell determinants (Fig. 1, *gcd*), is killed with a hot needle and these granules are thus prevented from taking part in embryonic development, the embryo produced by the rest of the egg lacks the germ cells. This supple-

ments former experiments in removing the germ cell determinants, and indicates that these granules really determine the germ cells.

2. When the primordial germ cells of *Leptinotarsa decemlineata* are killed in the blastoderm stage (Fig. 2, *pgc*) the resulting embryos lack germ cells. This is the earliest known stage in which surgical castration has been performed among the Insecta.

3. When the anterior or posterior parts of freshly laid eggs (Fig. 1) are killed, the material remaining alive develops that part of the embryo which it would have produced if the eggs had remained intact (Fig. 11). No regeneration of the part which would have been produced by the killed region takes place.

4. If the anterior or posterior parts of eggs in the blastoderm stage (Fig. 2) are killed, the resulting tissue represents the parts of the embryos which would have been produced by the living material if the entire egg had been allowed to develop (Figs. 8 and 9).

5. When parts of young embryos (Fig. 4) are killed, the remaining tissue develops normally (Figs. 15–18). Even small pieces of tissue (Fig. 17, *tf*), which are widely separated from the rest of the embryo, continue to develop normally.

6. Parts of old embryos develop up to the time of hatching. There is no regeneration of the killed part by the living tissue.

7. The eggs of *Leptinotarsa decemlineata*, at the time of deposition (Fig. 1), are definitely oriented with respect to the future position of the embryo.[1] The areas of the peripheral layer of cytoplasm (Fig. 1, *khbl*) are already set aside for the production of particular parts of the embryo, and if these areas are killed, the parts of the embryo to which they were destined to give rise will not appear. Likewise areas of the blastoderm (Fig. 2, *bl*) are destined to produce certain particular parts of the embryo.

THE UNIVERSITY OF MICHIGAN,
February 6, 1911.

[1] Hegner, R. W., '09, "The Effects of Centrifugal Force upon the Eggs of Some Chrysomelid Beetles." *Journ. Exp. Zoöl.* Vol. 6.

FURTHER EXPERIMENTS ON THE METHODS OF EGG-LAYING IN AMPHITRITE.

JOHN W. SCOTT.

INTRODUCTION.

On excursions, taken in connection with the course offered to students studying invertebrate zoölogy at Woods Hole, I have been impressed with the large number of forms not used for investigation. I believe this must be because little is known of their life-history and habits, because many of them are fairly abundant. With this idea in view, I recorded in a recent paper ('09) the results of some observations made upon the egg-laying habits of one of the marine annelids. In the form studied, *Amphitrite*, the germ cells arise in the typical way, *i. e.*, from the cœlomic epithelium. Very early these dehisce into the body cavity and mix with the cœlomic corpuscles. In some annelids as *Nereis*, the eggs escape by a rupture of the body wall. In *Amphitrite*, however, they pass to the exterior through certain nephridia that are highly modified to form gonaducts. In the paper mentioned above it was shown that *Amphitrite* deposit eggs at recurring periods that bear a close relation to the time of spring tides. It was further shown that at the time of oviposition, the body cavity contains floating free, not only the mature eggs and corpuscles but also the younger eggs in various stages of development. A single period of egg-laying occupies from 30–60 minutes, and most all eggs when extruded are in the metaphase of the first polar spindle. A few large, though unripe, eggs always escape especially toward the latter part of the period. In Fig. 1 is shown the comparative size and shape of various bodies found in the cœlome at this time. It will be noticed that the eggs when first set free in the cœlome are smaller than the red blood cells, yet neither cells nor eggs of this size ever escape during oviposition. Indeed, comparatively few of the large unripe eggs pass out through the nephridia. The

interesting question then arises, *How are ripe eggs separated from the other cœlomic bodies in the act of oviposition?*

References to Previous Paper.

At first an attempt was made to answer this question by studying the general anatomy of *Amphitrite*. In this group, as shown by Meyer, the septa are incomplete or absent, with one exception. The one complete septum, called the diaphragm, is near the anterior end and divides the cœlome into two unequal cavities. Anterior to the diaphragm the nephridia are for ex-

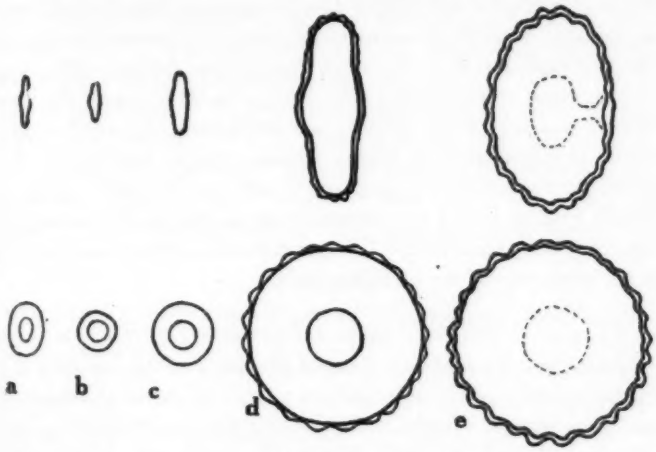

Fig. 1. To show the comparative size and shape of various bodies found in the cœlome of *Amphitrite* at the time of egg-laying; *a*, red blood corpuscles; *b*, youngest free egg observed; *c*, young egg, with little or no yolk; *d*, unripe egg, nearly mature; *e*, mature egg. Both edge and side views shown.

cretion only. The posterior nephridia are modified as gonaducts, and it is with these last that we are concerned. Their inner openings are bordered by large folded, or fimbriated, membranes, covered by strong cilia. Each opening connects with a large vascular sac, also ciliated. From these sacs tubes pass to the outer openings of the nephridia which are found in the species studied on segments 6–10 inclusive. From the dissections I came to the conclusion that there was no apparatus for sifting or "straining out" the ripe eggs.

However a theoretical explanation was given in which the nephridia were regarded as "settling basins," the cilia in this case preventing the settling of bodies which were not to be expelled. This explanation was based partly upon the structure of the nephridia, and partly upon the fact that "when the contents of the cœlome are stirred in sea-water, the largest ova including the ripe eggs, always settle more quickly to the bottom of the dish; the immature eggs then settle and last of all the cœlomic corpuscles." The important fact here is that gravity more quickly influences the large ova than the other bodies floating in the cœlomic fluid. It was suggested that the difference in the rate of settling was probably due to a greater density of the ripe eggs, but that the effect was possibly due to a difference in the shape of the bodies concerned. In either case, the conclusion was drawn that gravity is a differential means of separation. However, this left the matter inconclusive and the question arose, *Is this tendency of large ova to settle quickly due to a greater specific gravity, or to a greater mass in proportion to the amount of surface offering resistance?* To answer this was the primary object in the next step of the investigation.

Specific Gravity Experiments.

Having set the problem, I began the work of determining the specific gravity of the eggs and corpuscles found in the fluid of the body cavity. Incidentally the specific gravities of sperm, and of eggs in some later stages of development were obtained.

Lyon's plan of getting the density of eggs by centrifuging in gum arabic solution was tried and, after finding it suited my purpose, his method was adopted with slight modifications. First a strong gum arabic solution in sea-water was prepared and its density carefully determined. From this stock solution a series of stock solutions was made up of differing densities; this can be readily done by mixing with the stock solution in proper proportions sea-water of known density. When ready to centrifuge the capillary tubes of a hæmatocrit were filled about three fourths full of gum arabic solution. On top of these solutions whose density was known was placed the material whose density was required. In a few cases, in order to guard

against possible error caused by surface tension, the material was placed between two solutions of differing densities and then centrifuged. But surface tension did not appear to offer any appreciable resistance to the passage of the eggs and corpuscles either upward or downward. It was found that eighty turns of the hæmatocrit in one and one half minutes was sufficient to produce thorough separation of materials in the capillary tubes, without noticeably affecting the material within the egg membrane. This amount of centrifuging was therefore adopted throughout the entire series. I shall here present in order my findings in regard to (1) the specific gravity of eggs, (2) the specific gravity of corpuscles, and (3) the specific gravity of sperm, together with remarks as to the significance of these results.

After a number of preliminary experiments, in order to get familiar with the method to be used and to guard against possible sources of error, a series of tests was made upon eggs recently deposited. The results of some of these tests are shown in Table I. The female used in this series was found depositing

TABLE I.

To Show the Specific Gravity of Recently Deposited *Amphitrite* Eggs

Test No.	Density of Solution Used.	Material Used.	Result of Centrifuging.
25	1.090	Eggs just deposited.	Eggs on top.
26	1.085	Eggs just deposited.	Eggs on top.
27	1.080	Eggs just deposited.	A few begin to sink.
28	1.075	Eggs just deposited.	All went down.
29	1.0775	Eggs just deposited.	More than one half sink.
30	1.075	Eggs just deposited.	All on bottom.

eggs in a normal manner. Just as quickly as it could be done, while they were still being deposited, these eggs were centrifuged with the results shown in tests 25 to 30 inclusive. The table shows that the specific gravity of recently deposited, that is, mature *Amphitrite* eggs, lies between 1.075 and 1.085. They probably have a mean density very near 1.078; for almost all eggs remain on top of, and therefore are lighter than a solution with a specific gravity of 1.080, while more than half sink in a solution with density at 1.0775. Tests were made on eggs from other worms and verified the results given here.

Another series of tests was made in order to demonstrate the changes in density at different stages of development. See Table II. It was shown that very young eggs, those that had

TABLE II.

TO SHOW THE CHANGE IN DENSITY AT DIFFERENT STAGES IN THE DEVELOPMENT OF EGGS.

Test No.	Density of Solution Used.	Material Used.	Results of Centrifuging.
9	1.070	Cœlomic contents. Eggs half-grown and smaller.	Youngest eggs on top.
12	1 075	Cœlomic contents. Eggs nearly mature.	All eggs went down.
13	1.075	Eggs in various stages.	Unripe eggs on top. Ripe eggs down.
27	1.080	Mature eggs, just deposited.	Almost all on top, a few sink.
38	1.080	Same lot, after being in sea-water 1½ hours.	One half of eggs sink.
41	1 080	Same lot, fertilized. 8–16-celled stages.	One fifth of eggs sink.
47	1.075	Same lot, one hour later.	A few eggs sink. Most eggs partly down.
57	1.075	Same lot, trochophores, age 27 hours.	One third trochophores sink Best developed on top.

attained one fourth the size of mature eggs and in which there was not very much yolk laid down, had a specific gravity less than 1.070 (Fig. 1, *c*). In a word, the density of the egg as a whole is very noticeably increased as the yolk accumulates. It was also learned that allowing the deposited eggs to stand in sea-water for some time slightly increases their density (test 38). During segmentation and the formation of the segmentation cavity, the density grew less as one might expect (tests 41, 47). It probably continues to decrease slightly, at least as far as the late trochophore stage (test 57). My experiments did not carry the work further than this point.

Before the worm used in the first tests mentioned above, was through depositing eggs, the surface of its body was wiped dry and the cœlome was opened allowing the contents to escape into a clean glass dish. An examination with the microscope showed that the cœlomic fluid contained corpuscles and eggs in various stages of development, some being mature. Tests 32 to 37, Table III., show typical results in regard to the specific gravity of the corpuscles. Upon examining these results one finds that

TABLE III.

TO SHOW THE SPECIFIC GRAVITY OF CŒLOMIC CORPUSCLES.

The cœlomic contents were removed after the worm was about through depositing eggs.

Test No.	Density of Solution Used.	Material Used.	Result of Centrifuging.
32	1.080	Cœlomic corpuscles. Eggs in various stages.	Corpuscles sink. A few ripe eggs sink.
33	1.085	Cœlomic corpuscles. Eggs in various stages.	All corpuscles sink. All eggs on top.
34	1.090	Cœlomic corpuscles. Eggs in various stages.	Seven eighths corpuscles down. Eggs on top.
35	1.095	Cœlomic corpuscles. Eggs in various stages.	Most corpuscles sink. Eggs on top.
36	1.105	Cœlomic corpuscles. Eggs in various stages.	Two thirds corpuscles sink. Eggs on top.
37	1.123	Cœlomic corpuscles. Eggs in various stages.	One tenth corpuscles sink.
46	1.123	After standing three hours in sea-water.	Nearly one third corpuscles sink.

the specific gravity of the cœlomic corpuscles varies between wide limits. However, more than four fifths of them have a density greater than 1.090 and less than 1.123. Contrary to what my previous observations had led me to expect the corpuscles have a density greater than the mature eggs. All corpuscles are heavier and all eggs lighter than a density of 1.085. After standing in sea-water for some time the corpuscles appear to increase in density as shown by test 46, and by other tests not given. Furthermore, the smaller and what appear to be the younger corpuscles have a density less than the older ones. Under these circumstances an examination with the microscope after centrifuging was of course necessary to distinguish between the corpuscles. The significance of these results will be explained later.

In the course of my experiments the cœlomic corpuscles of both males and females were examined. While not suspected at the time, upon looking over my notes the rather curious fact came to light that the mean density of the female corpuscles is slightly greater than that of male corpuscles. I do not wish to emphasize this fact, for perhaps the number of individuals examined was not sufficient on which to base conclusions. Nor yet do I see whether the significance of these results pertains to nutrition

alone or to differences in reproduction, should they prove generally true for other annelids.

Another series of experiments showed the extreme lightness of the sperm; their specific gravity in sea-water being between 1.038 and 1.046. In contrast to eggs which increase in density as they mature, the sperm masses decrease in density as they grow larger and finally break up into free-swimming sperm. So that the density of the ripest sperm is even less than 1.038, as shown in Table IV. This density is interesting when considered

TABLE IV.

To Show the Specific Gravity of Sperm.

Test No.	Density of Solution Used.	Material Used.	Result of Centrifuging.
8	1.075	Contents of cœlome.	All sperm on top. All corpuscles sink.
40	1.075	Sperm shed in sea-water.	All sperm on top.
42	1.070	Sperm shed in sea-water.	All sperm on top.
43	1.062	Sperm shed in sea-water.	All sperm on top.
44	1.051	Sperm shed in sea-water.	All sperm on top.
45	1.038	Sperm shed in sea-water.	All sperm at bottom.
48	1.046	Sperm shed in sea-water.	All sperm on top.
50	1.041	Cœlomic contents of worm that deposited sperm on previous day.	All unripe sperm sink; a few nearly ripe on top.
52	1.046	Cœlomic contents of worm that deposited sperm on previous day.	Younger sperm masses sink. Older sperm masses on top.
55	1.051	Cœlomic contents of worm that deposited sperm on previous day.	Younger sperm masses sink.
56	1.038	Cœlomic contents of worm that deposited sperm on previous day.	A few lightest, ripest sperm on top.

in connection with fertilization and the habitat in which the animal lives. Water currents are undoubtedly important factors in the dissemination of sperm. But the sand flats on which these animals are found are securely protected from violent currents, where reefs or eel grass or shoals of adjacent islands break the force of the changing tide. The limited range of the animal does not require a wide scattering of the sperm. In fact the density of the sperm is such that, in the presence of very gentle currents, their distribution must be very limited at the time of oviposition. But the lightness of the sperm provides an easy

means for scattering them by currents over the sand flats, and their own locomotion aids them in their distribution. They have sufficient motor power to aid in horizontal distribution, and have sufficient density to prevent them from rising much above the bottom where the eggs are found. That sperm have such a method of distribution was substantiated in a different way by studying the scattering of sperm in dishes of still water. A large quantity of sperm was introduced at some point in the bottom of the dish; then the progress in distribution was observed by noting the advancing cloudiness of the water, reckoned in various directions from the point of departure. It was found that the advance in a general horizontal direction was many times more rapid than the advance in an upward direction. I take this to be due to the resistance of gravity. Since the sperm are set free near the bottom under normal conditions, their lightness tends to bring about more favorable chances for fertilization of the eggs.

We may now sum up the results of these specific gravity experiments in the form of certain explanations and conclusions:

1. The eggs of *Amphitrite* increase in specific gravity during growth in the cœlome. This is probably due to an increase in the amount of yolk. The reason therefore that the larger mature eggs settle in a dish of sea-water more rapidly than the smaller, immature ones, is undoubtedly due in part to the greater specific gravity of the older eggs. And in the process of egg-laying it is probable that a difference in specific density may act as a means to separate ripe from unripe eggs.

2. The reason why the larger eggs sink before the cœlomic corpuscles must be explained in another way, for the specific gravity of the corpuscles is decidedly greater than that of the eggs. The difference in behavior between these bodies is to be explained, I believe, by the flat, oblong shape of the corpuscles; their shape is such that they offer in settling a much larger resistance in proportion to their mass. Blood cells settle more slowly than eggs in sea-water because of a difference in shape and not because of a lesser density. Slight currents in the dish prevent the corpuscles from settling for a long time, while they hardly interfere with the downward movement of the eggs. At this

time in my experiments it was thought probable that ciliary action produces a similar effect in separating eggs and corpuscles during oviposition. This will be discussed later.

3. While the egg increases in density up to the time of oviposition it decreases slightly after fertilization, the decrease continuing at least as far as the trochophore stage.

4. The comparative lightness of the sperm is undoubtedly useful as an adaptation for scattering them at the time of fertilization. Their density is just sufficient to tend to keep them near where the eggs are to be found, but not enough to prevent a certain amount of locomotion.

Direct Observations.

During the past summer I was able to make a series of direct observations upon the separating process. In these observations I saw the ripe eggs pass over the fimbriated membrane, along the grooves, and finally into the vascular, nephridial sac ready to be expelled. At the same time the blood cells and unripe eggs were rejected and thrown back into the cœlomic fluid. The sight was indeed astonishing to see the general precision of a comparatively simple process.

It was by means of living dissections that the observations were made possible. First it is necessary to use only ripe females, and to determine this fact with certainty one needed to wait until the worm began to deposit eggs; then it was lifted out of sea-water and the surface of the body quickly dried. Next the cœlome was opened and its contents carefully drained into a clean watch glass; this was kept covered to prevent unnecessary evaporation while the nephridia were being removed. The worm was then placed in a dry dissecting pan and pinned at either end, so that it was held in a slightly stretched condition. The body cavity was opened by making a cut through the body wall in the mid-dorsal line; this cut began a little back of the middle of the body and extended forward to the anterior end. The enteric canal and accompanying blood vessels were next divided midway and their anterior portions removed. The flaps of the body wall on either side were then pinned out. In order to have these flaps spread out well, and so expose the large

nephridial sacs, it was necessary to cut the series of oblique muscles which extend from the body wall above the notopodial cirrus across the cavity to the body wall in the mid-ventral region. This species has five pairs of nephridia that function as oviducts. The cœlomic fluid of a ripe female contains some ripe eggs, many unripe ones, and thousands of corpuscles, mostly red blood cells. Each nephridium to be observed was removed entire and placed at once in the watch glass with the cœlomic fluid. Then the watch glass was placed on the stage of a microscope and the process studied.

As mentioned before the fimbriated membrane is a folded, or grooved structure covered for the most part with strongly developed cilia. The stroke of the cilia varies in direction and force on different parts of the membrane and their action furnishes the motor power that separates the eggs. By using a needle I could push near the fimbriated membrane any of the bodies I wished to study. The cilia are in continuous action, and their effective stroke, so far as I could observe, has a constant general direction. But the direction of the stroke varies on different portions of the membrane as follows: (1) On the edge of the membrane (Fig. 2, *a*) and near the entrance to the grooves between the folds, the stroke is such as to send currents toward or into the grooves. (2) In the grooves (Fig. 2, *b*), where the folds approach somewhat nearer to each other, the stroke at the bottom of the groove is still onward and inward; well up on the sides of the grooves the currents move upward and outward, and apparently with more force than at the bottom of the groove. (3) In the main groove (Fig. 2, *c*) the cilia at the bottom are arranged in approximately parallel lines that resemble small grooves, all leading inward and their action comparatively slow. Above on the sides of the pillars (Fig. 2, *d*), especially in the narrow grooves between pillars, the currents move rapidly and are directed upward and outward. With this explanation of the ciliary currents, both in regard to position and action, we may now consider how the different bodies are separated.

The Separation of Blood Cells from Eggs.—This occurs under the conditions of my experiment principally on the outer edges of the membrane. The currents are strong enough to lift and

draw in toward the membrane blood cells from a considerable distance; these cells therefore hit the cilia with some force, but the major stroke of the cilia is strong enough to bounce them off again, usually driving them away five to ten times their own diameter, and frequently beyond the limits of further attraction. However they may bounce two, three, or four times, that is,

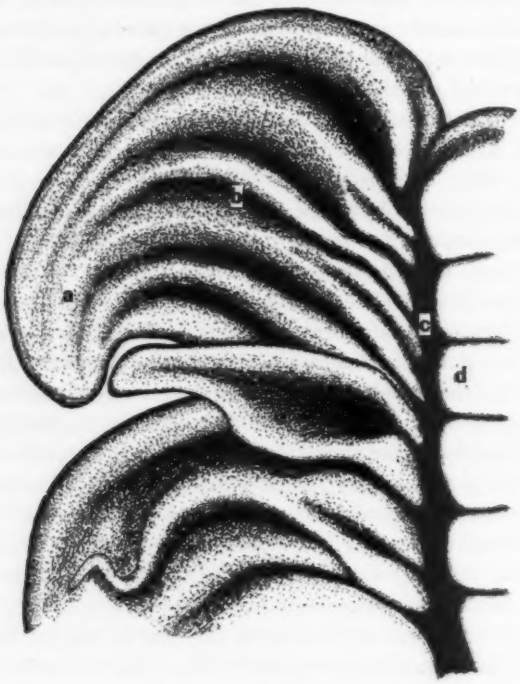

FIG. 2. To show a portion of the fimbriated membrane found at the inner end of a post-diaphragmatic nephridium of *Amphitrite*. Not so much magnified as Fig. 1. The membrane is undulating at *a*, and folding to form a groove at *b*; at *c* is the deep main groove leading into the nephridial sac; at *d*, are pillar-like projections overhanging the main groove. For further explanation see text.

until they reach the region of the groove shown at *b*, Fig. 2; then the cilia on the sides carry them up, out, and away. Only in very rare instances do blood cells ever reach the main groove; sometimes they follow the eddy near a mature egg, but they are

then thrown across the groove and against the pillars where the currents hurriedly lift them up and drive them away. I did not see a blood corpuscle pass down the main groove into the nephridial pouches, so long as the cilia retained their normal activity. After an hour or so their action becomes slower, but this is probably due chiefly to the fact that much plasma has evaporated.

The Separation of Immature from Mature Eggs.—The small, immature eggs, Fig. 1, *b* and *c*, as well as some of a larger size are eliminated in much the same way as blood cells. The task grows more difficult, however, as the eggs increase in size for they become too bulky to be bounced off the edge of the membrane like the blood cells. So, as the larger immature eggs (Fig. 1, *d*) are carried into the narrowing grooves, they are rolled up on edge, then lifted out and moved away. For when the sides of the groove narrow down so that both sides of the flat egg are affected by the currents produced by cilia on the opposite sides of the groove, the combined action of these currents is sufficient to lift even the weight of a large, immature egg. The case is different with the mature eggs, which *differ in shape*, are *more plastic*, and are *somewhat heavier* than most immature eggs found in the cœlome. These eggs are carried into the grooves down which they slowly pass, their shape and weight evidently being such that the lateral cilia are unable to lift them out of the groove. Undoubtedly their plasticity is also an important factor in resisting the action of the cilia. The side of the egg gives before the effective stroke of the cilia, so the result is to change the shape of the ripe egg rather than to push it away, and consequently such eggs move more slowly along the grooves. In some cases it was observed that the folds of membrane along the sides of the groove would respond to the presence of a ripe egg by folding over, virtually forming a closed tube. Inside this tube-like groove the egg could occasionally be seen changing shape, as it was forced slowly along toward the nephridial sac. When the membrane folded it was impossible for unripe eggs, or corpuscles on the membrane, to pass into the nephridial pouch with the ripe egg. The behavior of the membrane in the presence of a ripe egg had all the appearance of a tactile response,

and if any chemical reaction was here involved it could not be detected with the eye. The separation of these bodies appeared to be a purely physical process.

Since the cilia are continuously active, independent of any egg-laying period, it would seem that the egg-laying reaction is a direct response to changes in the egg that are chiefly produced by the breaking down of the germinal vesicle. As noted in an earlier paper a worm shows unusual muscular activity at the time of oviposition. This muscular reaction may be due to chemical changes in the body fluid, the irritation being produced by materials escaping from the nucleus. However another explanation is possible. As the ripened eggs begin to accumulate in the nephridial sacs, excretion, the normal function of these organs, would be hindered or stopped. This would lead to an accumulation of waste in the part of the cœlome posterior to the diaphragm, and in all probability would act as an irritant producing more vigorous muscular action until the eggs were expelled. In this way the wave-like movements of the worm at oviposition are a physiological response to an interference with excretion. Though these suggestions are largely theoretical, I am inclined to believe the latter view is the correct explanation.

Summary and Conclusions.

Amphitrite under normal conditions keeps up, with occasional brief resting periods, a series of wave-like contractions of the body for the purpose of sending water through its tube. A short time before oviposition the wave-like movements become stronger and slightly faster than usual. This excitement indicates the presence of ripe eggs and marks the beginning of the separating process. After some time has elapsed, and the nephridial sacs are well filled, each contraction wave as it approaches the anterior end of the body forces out through the pores small slimy streams of eggs. Oviposition usually continues from thirty to sixty minutes. The process of separation has been previously described. While the cilia furnish the motor power in separating the ripe eggs, strong wave-like contractions of the body wall furnish the pressure needed to expel the eggs from the nephridial pouches. It is suggested that this unusual activity of the worm

is probably due to interference with excretion, caused by clogging the nephridial sacs with eggs.

Undoubtedly much the same process occurs in other worms where eggs accumulate in nephridial sacs before oviposition. I am told by Downing that eggs accumulate before oviposition in the nephridial pouches of one species of *Arenicola*. Gerould has reported that the eggs of *Phascolosoma* collect in this way for several hours before they are laid, and there is little doubt that the same general method of egg-laying occurs in all worms with this habit. My results may be summarized in the following conclusions.

1. The separation of cœlomic corpuscles and unripe eggs, at the time of oviposition in *Amphitrite*, is accomplished by physical not chemical means.

2. The work of cilia on the fimbriated membrane furnishes the power used in the separating process. Wave-like contractions of the body aid in expelling the eggs, and it is suggested that these movements are due to interference with excretion, caused by clogging the nephridial sacs with ripe eggs.

3. The separating process is aided in several ways. The *shape* and *arrangement of grooves* on the fimbriated membrane, the *size* and *shape* of the bodies separated, and especially the *greater plasticity* and greater *density* of the mature eggs, all appear to be important factors in separating the bodies found in the cœlome at the time of oviposition. The action of the grooves in closing over a ripe egg is also a direct help in keeping other bodies from the main groove.

4. We are fairly safe in concluding that the method of egg-laying described for *Amphitrite* holds good for other worms where the eggs accumulate in nephridial sacs before or during oviposition.

NEMATOCYSTS OF MICROSTOMA.[1]

WILLIAM A. KEPNER.

For the past three autumns many brown *Hydras* and *Microstomas* were found in a fish pond northeast of the University of Virginia. In both these forms of animals were present oval, refractive bodies, which were the nematocysts. These nematocysts when everted have in both cases slender filaments with three barbules radiating from their base. The filament at its base is attached to the neck of a pear-shaped "poison-sack" or capsule. Since Oersted (1844) nematocysts were known to occur in flatworms as well as in Cœlenterata. Associated with the nematocyst of *Hydra* there is a cell which has elaborated the nematocyst and cares for it until it is discharged. This cell is known as a *cnidoblast* or *nematocyte*. Each cnidoblast of *Hydra* has a small spine-like structure—the cnidocil. This is supposed to receive the stimulus that results in the discharge of the nematocyst. When one examines the living *Hydra* and the living *Microstoma* found in this vicinity no difference can be detected between the nematocysts of the two forms, except that there is no cnidocil associated with the nematocysts of the flatworm. Moreover the nematocysts in both *Hydra* and *Microstoma* when undischarged and when being discharged appear to be normal parts of the respective animals. This indigenous appearance and ready discharge of the nematocysts of *Microstoma* led men to look upon them as structures elaborated by the cells of *Microstoma*. Such was my view after I had repeatedly, during three years, studied the nematocysts of living *Microstoma*. According to Martin (1908) until 1903 all zoölogists held that the nematocysts of *Microstoma* were its own products.

Recently the inference has been made that the nematocysts of *Microstoma* have been derived from ingested cœlenterata,

[1] The author wishes to thank Professor A. H. Tuttle, of this laboratory, Professor E. G. Conklin and Professor Ulric Dahlgren, of Princeton University, for valuable suggestions that were helpful in the preparation of this paper. This paper was read before the Philosophical Society of the University of Virginia.

as Grosvenor (1903) had claimed for the nematocysts of æolids. Martin (1908) must be given the credit for having first approached this question of the origin of the nematocysts of *Microstoma* in the proper way, *i. e.*, experimentally.

Martin first made the observation that *Microstoma* do ingest *Hydras*. "If a fasting *Microstoma* is placed in a watch-glass which contains some small *Hydra*, it is almost certain in a short time to come in contact with one of them. If the *Microstoma* comes suddenly against the tentacles of the *Hydra* it contracts itself immediately, and in this condition it may frequently be killed by the discharged nematocysts. As a rule, however, the *Microstoma* fixes itself for a short time by its posterior end in the neighborhood of the *Hydra*, and everts its pharynx to its full extent.

"The *Microstoma* then swims over the surface of the *Hydra*, usually attacking the lower part of its body with its pharynx fully everted (vide Fig. 10). The *Hydra* then usually becomes strongly contracted, and sweeps its tentacles over to the side on which it has been attacked, though under these conditions the tentacles do not grasp the *Microstoma*, but remain extended almost parallel with its body, and it would appear as though the pharyngeal secretion had a paralyzing action on the *Hydra*. In many cases, after a time, the *Microstoma* leaves its prey, and in such a case the *Hydra* does not seem much the worse for the attack, but if the *Hydra* is of small size, it may be engulfed and swallowed whole" (Martin, 1908, pp. 267–8).

After this observation Martin's chief evidence is based upon the histology of *Microstoma*. He finds that when both *Hydra* and *Cordylophora* are fed to *Microstoma* there are found within the tissues of the flatworm nematocysts peculiar to both kinds of cœlenterata that had been fed. Thus in sections of the *Rhabdocœle* he discovers nematocysts in the endoderm, mesoderm, and ectoderm. Martin's methods of procedure had convinced me that he had established that the nematocysts of *Microstoma* were derived from *Hydra*, until I had considered the details of his work.

I hesitate, for example, to follow Martin when he says that von Graff (1882) was wrong in saying that each nematocyst lies in a single cell and that "Hallez had correctly described the

nematocysts as lying in vacuoles" (Martin, 1908, p. 263). It had been the experience of students in my classes to find discharged nematocysts *enclosed within a single cell* (Fig. 1). Such isolated nematocysts were obtained by adding a drop of 1 per cent. acetic acid to a slide containing specimens of living flatworms. Some time after a cell of this kind had lain in the dilute acetic acid a large vesicle appears at the pole from which the discharged nematocyst must emerge (Fig. 2). Such distortion has frequently been observed under similar conditions.

So much for my observations upon the conduct and anatomy of living specimens and upon temporary preparations.

Before taking up the evidence afforded by permanent histological preparations I must interpolate my hesitancy, at least, to follow Martin when he says, "I have found very young *Microstoma* in which nematocysts were already present, and at first this presented a difficulty to this theory of the derivation of nematocysts, but I do not believe this is insuperable when we consider that nematocysts in the case of an animal which has fed largely on *Hydra* can be found in almost any tissue of he body. I have found them in the testes (Fig. 8), and although I have not yet found them in an ovum, I believe that the yolk-cells might readily carry them into the cocoon thus causing the infection of the young forms" (Martin, 1908, p. 271). It does not appeal to my judgment that nematocysts with cnidoblasts should be able to "infect" the young forms through the yolk-cells. Nematocysts *within* vacuoles, without attending cells to propogate them, *infecting* young forms through yolk-cells seem to be altogether improbable.

When I consider this last statement of Martin and note his oversight of the cells that in *Microstoma* do enclose some of the nematocysts I am led to believe that the final word has not been spoken concerning the nematocysts of *Microstoma*.

Such was my attitude when during September, October, November and early December, 1910, collections of *Microstoma* were made with a view to studying their histology.

Many specimens were fixed in chrom-aceto-formal; others were fixed in saturated solution of corrosive sublimate to which 5 per cent. of glacial acetic acid had been added. Both of

these fixing fluids gave good results. The sections were mounted serially. Some individuals were cut 3 microns, others at 4, 5, 6 and 7 microns. All the material was stained with Haidenhain's iron hæmatoxylin. Some sections were counter stained with Bordeaux red. From these many individuals a large list of sectioned nematocysts has been made.

As the specimens were being collected it was not an unusual experience to find greatly gorged specimens, which upon the addition of the fixing fluid would burst and thus liberate or expose a dead *Chætogaster* such as is represented in Fig. 3. In passing, this observation is of interest, for Martin says "one of the commonest enemies of *Microstoma* appears to be *Chætogaster*, which devours it greedily" (Martin, 1908, p. 268).

Within the enteron of the sectioned *Microstoma*, one frequently finds the large unbroken setæ of these ingested oligochætes (Fig. 4). If according to Martin, as I interpret him, the passage of the nematocysts from the enteron through endoderm and mesoderm to ectoderm is passive, I wonder why at times these harsh setæ of *Chætogaster* do not passively find their way into the tissues of the body of the flatworm. Other solid bodies such as diatom shells, and round or spheroidal objects such as the shells of *Arcella* lie within the enteron. These too, if the migration of the nematocysts be passive, should find their way to the surface as do the nematocysts; but not a single instance of such objects lying outside the lumen of the enteron was observed.

If, therefore, nematocysts do migrate from the cavity of the enteron to the general surface of the body, their migration seems to be accomplished through some active agency.

The first tissue of course to be concerned in this selective function is the endoderm. The cells of the endoderm must distinguish between the nematocysts and other solid or rounded objects within the enteron, just as an *Amœba* distinguishes between food and non-food. So far as the present series of observations is concerned twenty endodermal cells were found in which a nematocyst was enclosed (Fig. 5). In each case the nematocyst lies within a vacuole near the base of the endodermal cell.

The question at once arises, might not this nematocyst have wandered from the mesoderm into the endoderm? There is ample evidence of nematocysts migrating from one region to another in the bodies of cœlenterata. Schneider says: "Alle Nesselzellen der Siphonophoren enstehen an localisierten Bildungsherden, von denen sie in einen bestimmen Entwickelungstadium als Wanderzellen auf die Verbrauchstätten überwandern" (Boulenger, 1910, p. 764). In this connection "Hadzi's results are of the greatest interest, as he was able to examine living tissue as well as preserved material. His main conclusions are as follows:

"1. The thread-cells of hydroids are not formed 'in situ' but in the ectoderm of the cœnosarcal branches, where, on account of the thick perisarcal investment, they can obviously not become functional.

"2. When completely developed, except for accessory structures such as the cnidocils and the stalks, they migrate to the important nematocyst batteries on the tentacles. This migration can take place in two different manners. In simple forms, *e. g.*, *Campanularia*, the thread cells move actively by means of their pseudopodia, making their way between the ectodermal cells of the colony. In *Tubularia*, however, they adopt a quite different method of locomotion: from the ectoderm of the cœnosarc they force a way through structureless lamella and endoderm into the cavity of the hollow stem, whence they are carried by the current caused by the flagella of the endoderm cells to the hydranths. Here the thread-cells re-enter the tissues and migrate actively by their own movements to the ectoderm of the tentacles'' (Boulenger, 1910, p. 764). Conklin (1908) likewise observed the formation of nematocysts within the mesoderm of Actinia and their subsequent migration to the ectoderm. Boulenger states "that in *Mœrisia* the nematocysts of the oral battery of the medusa are developed in the endoderm at the base of the manubrium; this does not necessarily imply that the nematoblasts are themselves of endodermal origin" (Boulenger, 1910, p. 767). Thus we have much testimony as to the migration of nematocysts within the tissues of cœlenterata.

It is to be noted, however, that *in all these cited examples the*

nematocysts are transported while enclosed by cnidoblasts. In the twenty nematocysts found within the endoderm my material shows the stinging-threads lying free within vacuoles at the bases of the endodermal cells. Martin also describes the nematocysts that he noted within the endoderm of *Microstoma* as being free from nematocytes or cnidoblasts. In these cases, therefore, either the cnidoblasts after having carried the nematocysts into the endoderm have died or the stinging-cells were not taken into the endodermal tissue by cnidoblasts. Judging from what has been seen I am of the opinion that these nematocysts have found their way into the endodermal cells through the selective action of the latter. Such selective faculties have also been observed for the cnidophages of the ceras of æolids: Foreign bodies within the lumen of the cnidophore of æolids have been found "by Heckt, by Hancock and Embleton, and by Grosvenor. I have found uninterpretable fragments of tissue, bodies not tissue-like, and diatoms, in the liver diverticula and in the cnidophores. The ciliated canal has no power to distinguish other indigestible bodies from nematocysts, but this inability to select is not shared by the cnidophages, for so far as I know, these ingest only nematocysts" (Boulenger, 1910, pp. 127–8).

The cnidophages of æolids deliver their nematocysts to the cnidocyst, whereas the endodermal cells of *Microstoma* deliver their nematocysts to the mesoderm.

In the material of this laboratory numerous nematocysts are found within the mesoderm. The nematocysts in the mesoderm that lie nearest the endoderm are in all cases enclosed in a vacuole. These vacuoles are similar to those described and figured by Martin and Vallez. About each vacuole the mesoderm crowds. Two kinds of cells of the mesoderm are to be found in or near the walls of these vacuoles: (*a*) a branched type of cells, which form the frame-work of the mesoderm. These cells have nuclei whose chromatin granules are conspicuous but more or less equal in size. The cytoplasm of this first type of cell is not very dense and is greatly branched, its branches anastomosing with those of its fellows (Fig. 6, A); (*b*) cells which are much less frequent in their occurrence, with denser, more compact cytoplasm than that of the first type of cell, the nuclei of the second kind of cells being

characterized by having a relatively large black nucleolus which lies in a vacuole (Fig. 6, *B*). These are taken to be the wandering cells or amoebocytes of *Microstoma*.

When the nematocysts are found lying nearer the ectoderm cytoplasmic strands appear extending more or less into the vacuoles about the nematocysts. At *P*, in Fig. 6, is represented what appears to be pseudopodia of a wandering cell invading a vacuole.

Whether or not in this and similar vacuoles there is evidence of amoebocytes entering the vacuoles, the *vacuoles* in all cases are filled by a cell when the nematocysts come to lie near the ectoderm. About the nematocysts that have but recently reached the ectoderm the cytoplasm and nucleus of the invading cell are much more evident than in the cases where the nematocysts have been at the surface sufficiently long or near to suffer discharge (Figs. 7 and 8). In other words the cells seem to spend their vitality in caring for these exotic nematocysts and about the partially discharged nematocysts are to be found cytoplasm and nuclei which indicate a depleted condition (Figs. 9 and 10).

The nuclei of those cells do not lie at the surface of the vacuole as Martin shows in his figure number four, plate 14, as he describes in his legend for this figure. The nucleus in each case can be seen to lie well within the lumen of the original vacuole. This may be determined by the study of transverse or longitudinal sections. Figs. 7 and 8 represent two contiguous sections of a nematocyst near the ectoderm. Fig. 7 was taken through or near the equator of the nematocyst. Fig. 8 represents the section taken immediately following that shown in Fig. 7. In the eighth figure the lower surface of the section is shown. When the focal plane is raised above this level three microns the nuclei of the overarching mesodermal cells appear and the nucleus of the invading cell disappears. Thus it is determined beyond doubt that there is intimately associated with the exotic nematocysts, when they come to lie near the ectoderm, a cell which has invaded the vacuole, that had appeared about the nematocyst within the endoderm. This cell can be seen in fresh material as described earlier in this paper and in all of my permanent preparations.

The question naturally presents itself as to what kind of cell it is that has thus entered the vacuole. In all favorable nuclei that are found within the vacuole there is present a deeply staining nucleolus about which there is to be seen a vacuole (Figs. 8 and 10). This makes the nuclei more closely resemble those of the amœbocytes than those of the other mesodermal cells of the *Microstoma*. So I conclude that in each case an amœbocyte enters the vacuole to take charge of the nematocyst as do the cnidophages of the æolids.

It is interesting to observe how these nematocysts are handled by the invading cell of each vacuole.[1] The nematocysts when they lie alone within the vacuoles are pointing indifferently in all directions, but after they have been taken charge of by the cnidophages they have in all instances their discharging poles directed towards the exterior of the body. Martin also observed this final exact orientation of the nematocysts. He says of it "the large barbed nematocysts in their final position, always lie so that the thread, when it is discharged, will pass out of the animal, although they may lie pointing in any direction while they are still in the gut cells or the body-cavity. This rule does not seem to hold good in the small cylindrical nematocysts, which, as far as I can see usually lie almost parallel to the surface. It is very difficult to say how such an orientation can be effected, but something of the same kind has been detected in *æolids*, and I believe that the same difficulty is present in the nematocysts of the tentacles in cœlenterates" (Martin, 1908, p. 267). It would indeed be a most difficult matter to understand their orientation if Hallez and Martin are correct in describing these exotic nematocysts as always lying within vacuoles. The cnidophages appear to be the vital agents which properly orient the nematocysts.

In my preparation one barbed nematocyst was observed that had been apparently inaccurately discharged. This was taken from near the middle of a specimen that when fixed remained extended. In such distended specimens the mesoderm near the middle of the animal is reduced to a very thin layer; this together

[1] Hereafter we shall speak of this invading cell of the vacuole as a "*cnidophage.*"

with a possible shifting of the mesoderm at the exact time of discharge may reasonably account for this single exception that is represented in Fig. 11.

There is a second type of nematocyst found in *Hydra*. These are the so-called "grappling" nematocysts. By means of them the *Hydra* lays hold of or entwines objects of prey; when the prey is thus seized the tentacles of *Hydra* sweep it into the mouth of the polyp. This seems to be the chief function of these nematocysts; but the poison sac at the base of this type of nematocysts suggests that they are something more than mere prehensile structures. If they are not more than this, I wonder at *Microstoma* taking them into its tissues and passing them out to its surface where they are, so far as my observations are concerned, always oriented as are the barbed nematocysts. Frequent nematocysts of this type have been found by me within sections of *Microstoma*. In some cases these grappling nematocysts lie within vacuoles in which no attending cell or cnidophage can be recognized. Fig. 12 shows such a nematocyst lying near a vacuole from which it may have been discharged. These nematocysts are small and their vacuoles likewise small so that it is difficult to determine whether cells, associated with them, lie within their vacuoles or not; but frequent nematocysts of this type have been found that have attending cnidophages within their vacuoles (Fig. 13). Moreover all the exotic "grappling" nematocysts were so oriented that their discharging poles were directed towards the surface.

I have come to look upon the nematocysts of *Microstoma* as being derived from *Hydra*. This conclusion is based upon the following facts: (1) that there is a great variation in the number of nematocysts of *Microstome*—from very few to many; (2) the close resemblance between the nematocysts of *Hydra* and *Microstoma*; (3) the absence of cnidocils in *Microstoma;* and (4) the absence of cnidoblasts about the nematocysts when found within the endoderm and deeper mesoderm of *Microstoma*.

Toward what end is all this careful handling of the exotic nematocysts of *Microstoma* directed?

Glaser (1909) has determined by experiment that nematocysts will not be discharged in the digestive fluids of æolids.

There would be no protection gained by the flatworm in passing them into the tissue of its body if in the digestive fluids of *Microstoma* the nematocysts are likewise not discharged; and in case they did discharge within the digestive fluids of *Microstoma* they would have done their injury before they had been taken up by the tissues of the *Rhabdocœle*.

Glaser (1909) has further demonstrated that when nematocysts pass from digestive fluids into fresh water in all cases they discharge. Here then are seen grounds for making the inference that his careful handling of the nematocysts is done in order to prevent their haphazard discharge in the region of the mouth as they are egested. Over against this inference is the fact that in æolids certain nematocysts pass from the alimentary canal with the waste food while others are more carefully handled and discharged by the cnidophages of the cerata (Glaser, 1909): also when a flatworm is bold enough to attack a living *Hydra* with its anterior end it can hardly be considered to be so serious a matter to have nematocysts discharged casually within the vicinity of the mouth.

Again the end may be to avoid discharge of nematocysts by means of distortion within the enteron; for Glaser (1909) has shown that nematocysts when distorted do discharge. This suggestion I think can be dismissed with the inference that within the mesoderm the nematocysts are just as liable to distortion as within the enteron; for through the muscular actions of the body the ova are seen at times to suffer considerable distortion.

The third suggestion is that these bodies are taken up to be used. Grosvenor says: " 'No one can have witnessed the reaction of an æolid to various stimuli . . . without being convinced that the cerata are used as a means of defence' " (Glaser, 1910, p. 138). Likewise no one can have witnessed the discharge of nematocysts of *Microstoma* when stimulated by pressure or by acetic acid without looking upon them as organs of defense.

It taxes my faith seriously to believe that endodermal cells select nematocysts for so remote a purpose as that of defending the cell-colony at the ectodermal surface, and yet until further evidence can be obtained, if I must take a stand, I conclude that these exotic nematocysts are handled by *Microstoma* for purposes of defense.

If this conclusion is correct and if the nematocysts of æolids are derived from cœlenterata, the question can be raised, among many other more subtle ones, have the æolids acquired their method of dealing with nematocysts of cœlenterata through flatworm ancestry, or are these cases of parallel development of reflexes or instincts?

I hope to be able to make a series of experiments upon the nematocysts of these *Rhabdocœles*.

SUMMARY.

The nematocysts of *Microstoma* are derived from *Hydra* upon which *Microstoma* feeds. These ingested nematocysts are delivered to the mesoderm by the endoderm of the flatworm. Within the mesoderm an amœboid cell takes charge of each nematocyst and as it is transported to the surface orients it so that the "sting-thread" has its discharging pole directed towards the exterior of the animal. Thus *Microstoma* has come to use the nematocysts of its prey as do the æolids use the nematocysts of their prey.

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March, 1911.

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EXPLANATION OF PLATE I.

FIG. 1. Nematocyst with its associated cell. Obtained by gentle pressure of cover-glass upon a specimen treated with a drop of 1 per cent. acetic acid. Free hand drawing, with aid of micrometer eye-piece. ×1,800.

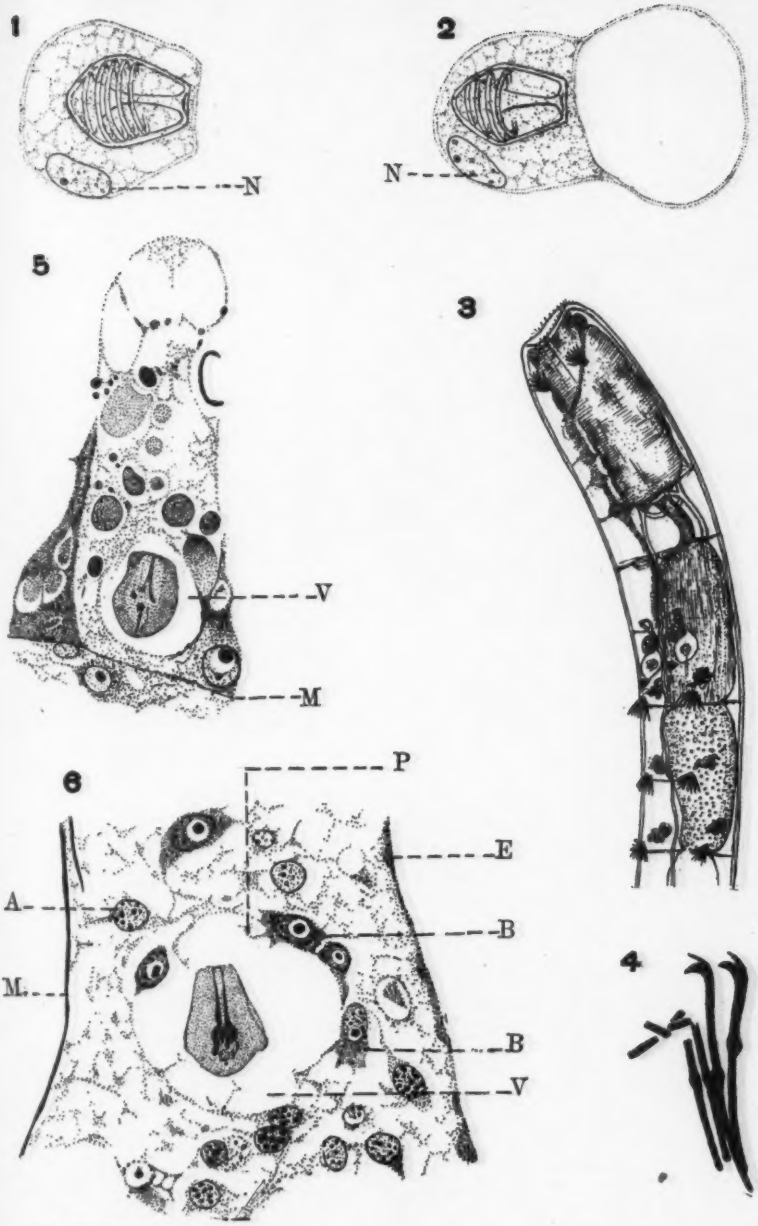
FIG. 2. Same cell after lying ten minutes in the dilute acetic acid. Vesicle has formed at one end of cell. Free-hand drawing, with aid of micrometer eye-piece. ×1,800.

FIG. 3. Anterior end of *Chætogaster*. Free-hand drawing.

FIG. 4. Setæ taken from lumen of enteron of sectioned *Microstoma*. Camera lucida drawing. ×500.

FIG. 5. Endodermal cell. Nematocyst lying within vacuole, *V*, at base of cell; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 6. Nematocyst within vacuole, *V*, lying near middle region of mesoderm; *A*, supporting cell of mesoderm; *B*, wandering cells of mesoderm; *E*, ectoderm; *M*, basement membrane of endoderm; *P*, pseudopodium (?) of wandering cell. Camera lucida drawing. ×1,500.



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EXPLANATION OF PLATE II.

FIG. 7. Undischarged nematocyst with its vacuole filled by cytoplasm. It lies near the ectoderm, *E*. Camera lucida drawing. ×1,500.

FIG. 8. Section next to that represented in FIG. 7. Shows the nucleus, *N*, of cell associated with the nematocyst. Camera lucida drawing. ×1,500.

FIG. 9. Partially discharged nematocyst. *E*, ectoderm; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 10. Partially discharged nematocyst. *N*, nucleus of cell associated with nematocyst. Camera lucida drawing. ×1,500.

FIG. 11. Nematocyst discharged approximately parallel to the surface. *E*, ectoderm; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 12. Grappling nematocyst, partially discharged. *E*, ectoderm, *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 13. Grappling nematocyst, partially discharged. An attending cell or cnidophage is shown within the vacuole within which the nematocyst lies. Camera lucida drawing ×1,500.

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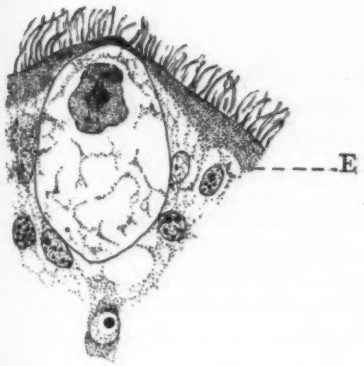

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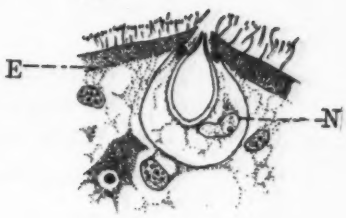

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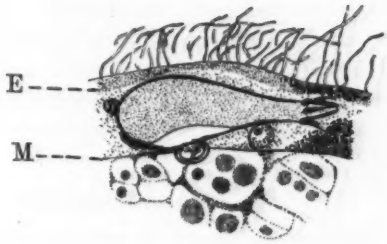

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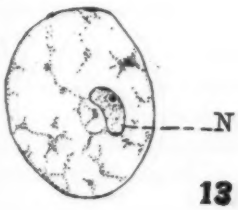

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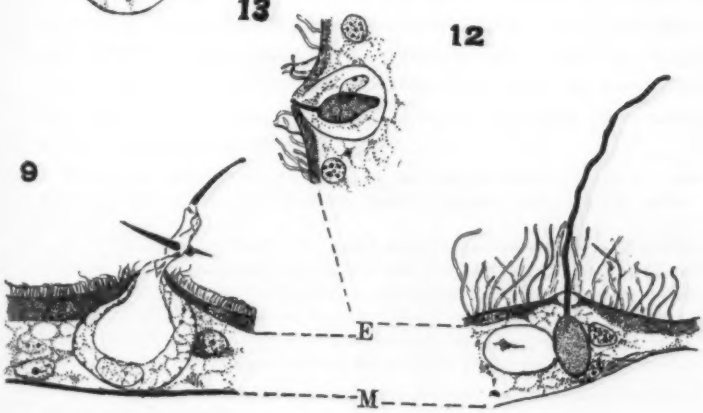

W. A. KEPNER

EXPERIMENTS ON THE ADOPTION OF LASIUS, FORMICA AND POLYERGUS QUEENS BY COLONIES OF ALIEN SPECIES.[1]

MAURICE COLE TANQUARY.

The experiments recorded in this paper were performed during the past summer in the laboratory of the Bussey Institution, Harvard University, at the suggestion and under the direction of Professor W. M. Wheeler. At the beginning of this particular series of experiments I had in mind only two species with which I intended to work, namely, *Aphænogaster tennesseensis* and *Aphænogaster fulva* with its subspecies *aquia* and variety *picea*, but a number of field excursions taken during July and August brought to hand several other species which served as such excellent material for similar experiments that it was decided to include them also.

Object of Experiments.

The object of these experiments was to determine whether the queens of certain species of ants are parasitic upon certain other species in founding their colonies. The various methods employed by queen ants in founding their colonies are stated in Wheeler's "The Founding of Colonies by Queen Ants," and since the following experiments have a direct bearing upon that question, and in fact form a continuation of exactly the same sort of work, I think I can do no better in the beginning than quote a few paragraphs from that paper.

"Female, or queen, ants in founding their colonies resort to one of three methods, which may be known as the usual or typical, the redundant, and the defective. . . .

1. "The female ant is able by herself alone to start her colony; that is, under favorable circumstances she can produce and bring to maturity the first brood of workers and thus insure the further growth and development of the colony. She is capable of passing many months without nourishment even while she is feeding her off-spring. Her voluminous fat-body, built up during her larval life in the maternal nest, together with her degenerating wing-muscles, furnish the sub-

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 29.

stances that are converted into food for the young. Although so arduous that few of the many queens of all that celebrate their nuptial flight during a season ever succeed in founding a colony, this method is, nevertheless, the one adopted by the great majority of ants."

The second method refers to the fungus-raising ants (Attii) and need not be given here.

"3. The female ant, owing to her small and delicate stature, or delayed fertility, is quite unable to found a colony without the aid of workers of another species. This method, which is resorted to by parasitic species—using that term in a very broad sense—appears under three different aspects.

"A. As temporary social parasitism. The female seeks and obtains adoption in a small queenless colony of another species and permits its alien workers to bring up her young. When these have matured, they emancipate themselves and become an independent colony, either by emigration or, more probably, only through the natural death of the host species."

"B. As permanent social parasitism. The female seeks and obtains adoption in a colony of some other species and there permanently resides together with her offspring. Examples: *Anergates*, *Strongylognathus*, *Protomognathus*, *Wheeleria*, etc.

"C. As dulosus, or slavery. The solitary female enters a small colony of another species, kills the workers, and seizes and rears the progeny (larvæ and pupæ) as a first step towards bringing up her young. The workers produced by the female subsequently make forays on other colonies of the host species and appropriate their offspring. While they use a portion of these as food they permit another portion to develop as 'auxiliaries' or 'slaves' so that the colony preserves its 'mixed' character."

In the same paper and in others Wheeler has also brought out the fact that parasitic species are sporadic in their occurrence, that they usually produce a very large number of females which are aberrant in some way, such as being of unusually small size, sometimes smaller than the workers, in being mimetic in coloration or behavior, in being covered with fulvous, myrmecophilous hairs, etc. Therefore the possession of the above characteristics to any extent by a species of ant is good grounds for believing that the queens of that species may be parasitic in founding their colonies.

A paragraph on page 447 of Wheeler's book, "Ants, their Structure, Development and Behavior," gives the reasons for performing adoption experiments with queens of *Aphænogaster tennesseensis*.

"The female of the myrmecine ant *Aphænogaster tennesseensis* in being deep red and of very small size, with a glabrous body and huge, flattened, epinotal spines protecting the vulnerable abdominal pedicel, is so unlike the females of any other members of the genus *Aphænogaster* that she was originally described by Mayr as the type of a distinct species (*A. levis*). These peculiarities suggest temporary parasitism and this is borne out by the observations of Schmitt

and myself (1901*c*). Schmitt found near Beatty, Pa., a small mixed colony of *A. tennesseensis* and *A. picea*, a variety of *fulva*, and one of the commonest ants in the northern states. He was impressed with the fact that the nest of this colony was under a stone, because *tennesseensis* normally nests only in rotting wood. During the summer of 1902 I found near Rockford, Ill., two mixed colonies like that observed by Schmitt, except that the variety *picea* was represented by the variety *rudis*. Both colonies were of small size and situated under stones. In one of them a *tennesseensis* queen was unearthed. There can be little doubt, therefore, that the glabrous queens seek out small nests of some variety of *fulva* and start their colonies in them just as *consocians* does in the nest of *incerta*. This habit is also indicated by the sporadic distribution of *tennesseensis* and its occurrence only in localities where some form of *fulva* is abundant."

The reasons for performing adoption experiments with queens of the other species will be given as I take up those experiments.

Method.

The method used in these experiments consisted in placing artificially dealated queens of the supposedly parasitic species in a nest with a few workers and brood of the supposed host species and observing their behavior under these conditions. This is the same method used by Wheeler in the experiments recorded in the paper mentioned above, and possible objections to it are answered by stating that an artificially dealated queen behaves in the same manner as a fertilized queen that has dealated herself, and that if one is to conduct such experiments on a large scale it is necessary to use artificial nests and to prevent the escape of the introduced queen. For most of the experiments I used large Petri dishes 4 1/2 and 6 in. in diameter into which I dumped the ants with their brood and some earth from the nest. For some of the experiments, however, I used nests of the Fielde pattern, and in that case I always introduced the queen into the light chamber and allowed her to find her own way into the dark chamber where most of the ants stayed, thus imitating natural conditions a little more closely. When I used Fielde nests, one chamber was kept covered with a piece of orange-colored glass. A few of the experiments with each species I watched very closely, especially when the queen was first introduced, sometimes watching a nest almost constantly for several hours, but as I had a large number of experiments going at the same time, sometimes more than fifty, I had to be content with examining most of them hastily several times a day and depending

chiefly upon the final result which, after all, is the important thing.

APHÆNOGASTER TENNESSEENSIS.

As this species does not occur in eastern Massachusetts I collected part of a large colony from a wood near Urbana, Ill., June 8 and brought it with me a few days later to the Bussey Institution, where I transferred the ants to a plaster of Paris nest, made on the Fielde pattern. The nest contained only workers and a large number of larvae. By the middle of July winged females began to appear and during the summer more than a hundred were produced. These, and a few sent me from Illinois later in the summer by Messrs. Hugh Glasgow and R. D. Glasgow, were the ones used in the experiments. Altogether I tried thirty-five queens of *A. tennesseensis* with eleven different colonies, two of which were *A. fulva* subspecies *aquia* var. *picea*, one *A. fulva*, and the others *A. fulva aquia*. The latter is probably more common where *A. tennesseensis* occurs. Of these thirty-five queens I got but one clear case of adoption and unfortunately this was with one of the colonies for which I have but scanty notes. The notes of this experiment are as follows:

EXPERIMENT B. 24c.

Aug. 18.—3.00 P.M. I place an artificially dealated queen of *Aphænogaster tennesseensis* in a Petri dish with five workers and about fifty pupæ of *A. fulva aquia*.

Aug. 19—9.00 A.M. Queen standing by herself.

Aug. 20—9.30 A.M. The same.

Aug. 21—11.30 A.M. She is standing on the pile of pupæ with the workers, none attacking her.

Aug. 22. The same.

Aug. 23—8.30 A.M. She stays in the midst of the workers and pupæ and seems to have been adopted.

Aug. 23—3.30. Still with the workers—they never attack her.

Aug. 24—8.00 A.M. In the center of the bunch of workers; about three dozen of the pupæ have become callows.

Aug. 28. Still being treated as their own queen.

Sept. 10. I have examined her every day up to the present time, and have never seen her treated by the workers other than as their own queen. There are now about sixty workers.

In all the experiments with *Aphænogaster tennesseensis* the queen was attacked by the workers, usually at the very outset, sometimes not until she had been in the nest for a few minutes.

Her behavior was about the same in most cases; she was timid and either ran away or crouched down in a supplicatory posture. Some of the queens, however, showed no fear whatever at first and approached the *fulva* workers as though they were members of the same colony. Constant biting and pulling about by the legs and antennæ, however, soon caused them to try to escape from their tormentors. In nearly every instance the behavior of the introduced queen was conciliatory and in no case did she at first seem to resent her harsh treatment but seemed to try either to conciliate the *fulva* workers or to escape from them. One queen, however, as will be shown in the following experiments, departed widely from what is apparently the normal behavior under such conditions by seizing a worker and carrying it about when the others attacked her. At first this behavior seemed to render her almost immune from further attacks and I began to think that perhaps only such queens as showed this particular phase of behavior succeeded in getting themselves adopted. Later on, however, the attacks were renewed and the queen was finally killed. I will give one experiment in detail as a type, and summarize the others.

EXPERIMENT B. 18.

Aug. 9—9.00 A.M. I place an artificially dealated queen of *Aphænogaster tennesseensis* in the light chamber of a Fielde nest containing about two dozen workers and a small pile of larvæ and cocoons of *A. fulva aquia*. She goes at once into the dark chamber. The first few workers that she meets recognize her at once as a stranger, but do not attack her. Several threaten her and then pass on. After about five minutes one worker grabs her by the petiole and carries her about in the nest. She does not seem afraid and does not try to get away; her manner is conciliatory.

9.10 A.M. She goes out into the light chamber but soon returns.

9.20 A.M. One worker is tugging at her antennæ.

9.25 A.M. The same worker is still holding her.

9.27 A.M. She gets free from the worker and runs around in the dark chamber. Many of the ants she meets do not attempt to attack her.

9.30 A.M. Another worker catches her by an antenna and holds her for a while.

9.44 A.M. Two workers lick her for about two minutes and then go away.

9.47 A.M. A worker seizes her by one of her tarsi and pulls her about for a while.

9.48 A.M. Three workers are licking her. She is resting on the sponge about one half an inch from the brood. A number of workers keep passing by her; some of them stop and lick her a while, some feel her over with their antennæ, open their mandibles as though to attack her and then go away without doing so. Sometimes she pats the heads of the workers with her antennæ as though begging food.

10.09 A.M. One worker catches her by a middle leg and pulls her about for a minute, then stops and licks her thorax and abdomen, then seizes her again by the leg and pulls her, but apparently not very hard. Again it stops and licks the queen for a minute and then goes a little to one side and begins cleaning its own antennæ but soon comes back to the queen and repeats the whole performance. The worker keeps this up for twelve minutes and then goes away. The queen has remained all this time in the same position, one half inch from the brood.

2.00 P.M. She is out in the light chamber but soon goes back.

4.35 P.M. One ant holding her by the petiole, another by one antenna.

6.00 P.M. A worker holding her by the antennæ.

8.15 P.M. The same.

Aug. 10—7.15 A.M. She is out in the light chamber alone.

9.15 A.M. She is in the dark chamber but not with the workers.

11.30 A.M. She has been in the light chamber by herself most of the morning.

11.50 A.M. Two workers are pulling her about.

2.00 P.M. She is running around in the light chamber, trying to get out. The workers seem more hostile today than yesterday.

Aug. 11—8.00 A.M. The queen is dead; I remove her.

9.05 A.M. I place another queen in the light chamber. She starts into the dark chamber and is seized in the passage way by two of the workers, one pulling an antenna, another holding her petiole. They drag her about in the dark chamber.

9.50 A.M. The queen is on the sponge near the brood and has seized the petiole of one of the workers in her mandibles. Workers are standing about but do not attack her; once in a while a worker licks her.

10.30 A.M. A worker seizes her by the hind tarsus and holds her for five minutes. She is still holding the worker.

10.15 A.M. Still in the same place. She is straddling the head and thorax of the worker which she is holding by the petiole. Once in a while a worker seizes her by a leg or an antenna, but some of the workers are licking her.

10.30 A.M. Still holding the worker.

11.10 A.M. The same.

12.10 P.M. The same.

1.30 P.M. The same. She is standing in the midst of the brood.

3.50 P.M. Still holding the worker; she is carrying it around now on the sponge and in the midst of the other workers and brood. She pays no attention to the other workers, some of which nab her at times. There is no excitement in the nest.

4.30 P.M. Still carrying the worker around; she does not get far from the sponge and most of the time is on it with the brood and workers. The worker seems to be nearly dead and for several hours has made no effort to escape or defend itself.

5.00 P.M. The same.

6.00 P.M. The same. A worker holding the queen by the antennæ.

8.00 P.M. The queen is still on the sponge holding the worker.

9.00 P.M. She is about two inches from the sponge, a worker holding her by the antennæ; she is not holding the worker now.

Aug. 12—8.00 A.M. She is again on the sponge with the brood, a worker tugging at one of her tarsi.

9.00 A.M. She is about two inches from the sponge, not holding the worker, but one of them is holding her by the antennæ.

9.15 A.M. She is again on the sponge with the brood and is holding the worker by a middle leg. The worker seems to be helpless in her grasp and does not attempt to escape.

9.22 A.M. I change the orange glass in order to make the other chamber the dark one.

9.35 A.M. The brood has all been removed to the moist sponge in the other chamber. A few ants still running about in this (now light) chamber. The queen and her victim still on the sponge. The other ants sometimes come up and feel them over with their antennæ but do not attack the queen.

10.00 A.M. During the past forty-five minutes the queen has been standing almost motionless. She now begins to move about on the sponge, probably because all the other ants are gone. At times she stands on her middle and hind feet and with her front feet seems to try to twist or change the position of the worker she is holding. She bends under her abdomen as though spraying the worker with formic acid.

10.10 A.M. She leaves the sponge and carries the worker about for a minute and then comes back.

11.30 A.M. She has remained on the sponge all this time holding the worker.

11.40 A.M. She is alone on the sponge. I do not know whether the worker escaped or whether it died and she dropped it. There are several dead workers in this chamber.

11.45 A.M. She is running about in the light chamber and seems to be entirely uninjured.

11.50 A.M. She goes into the dark chamber, seizes a worker by the thorax near the petiole (almost exactly the same hold she had on the first worker) climbs on to the sponge and stops just in the middle of the brood and workers some of which pull her legs or antennæ, but she retains her hold on the one worker and remains in the same position. I remove all the dead workers from the nest.

1.40 P.M. Still holding the worker in the same way.

3.15 P.M. The queen is still holding the worker and is staying with the brood.

6.15 P.M. The same.

Aug. 13—9.15 A.M. The same. There are no dead workers in the nest so she has not killed any of them.

11.00 A.M. The same.

12.00 M. The same.

1.25 P.M. The queen is not holding the worker but three of the workers are holding her, one by the petiole, one by an antenna and one by a mouth part.

3.00 P.M. Three workers are holding the queen.

6.00 P.M. The queen is again holding a worker and three workers are holding her.

Aug. 14—9.30 A.M. The queen is being stretched out by two workers that are pulling in opposite directions on her legs. She is not holding the worker now.

10.30 A.M. The queen is running about in the light chamber.

6.00 P.M. She is alone in the light chamber, and has two legs and one antenna partly gone.

Aug. 15—9.00 A.M. In the light chamber alone; she has four legs and both antennæ partly gone.

1.50 P.M. The same.

3 50 P.M. The queen is dead and dismembered.

Aug. 16—8.15 A.M. I place in another queen. She runs about in the nest avoiding at first the workers who threaten her. After about four minutes when she is resting on the sponge (not with the brood), a worker comes up and begins licking her at once and continues doing so for more than five minutes.

8.45 AM. The queen is in the corner of the nest feigning death. I thought she was dead at first; several workers standing around her.

9.00 A.M. She gets up and goes over to the brood; after a few minutes a worker drags her away.

9.30 A.M. Three workers holding her.

12.00 M. They are still attacking her.

3.00 P.M. She is being stretched out by four workers.

6.00 P.M. The same.

Aug. 17—7.30 A.M. Three workers are holding her. One leg partly gone.

5.30 P.M. The queen is dead and dismembered.

On August 19 I placed in another queen. She received the same treatment and was killed by August 24. On August 25 I placed in another. This one fared a little better and lived until September 8.

This colony, it will be seen, killed in succession five queens of *Aphænogaster tennesseensis* between August 6 and September 8. That there is a tendency toward adoption is seen in the fact that a number of the workers often licked the queen just as they would their own, while others were attacking her. It seems as though there is something about the queen that is attractive to the workers while at the same time the odor of a different species antagonizes them. In nature the specific odor is probably not so distinct by the time the *tennesseensis* queen enters a *fulva* nest since she will have been out of the parent nest and running about on the ground for some little time. In this and in other adoption experiments only a few ants attacked a queen at once, usually not more than two or three, and very seldom more than five or six, even when the colony was a very large one. It would seem as though the other ants realized that the intruder was being taken care of.

The variety *picea* seemed to be more hostile to the *tennesseensis* queen than *aquia*, but even here a tendency toward adoption is shown, as may be seen from the treatment accorded one of the queens in an experiment from which I take a few notes. This colony contained seventeen workers and a small pile of larvæ of *Aphænogaster picea* and were in a small two-chambered Santschi nest made of glass and plaster of Paris.

July 25—2.45 P.M. I place in the first queen. She receives very harsh treatment and is killed by 4.00 P.M. and her body cut in two at the petiole.

4.10 P.M. I place in a winged queen. She receives no better treatment than her predecessor and is killed by 6.00 P.M.

July 27—9.15 A.M. I place in the light chamber another artificially dealated queen of *Aphænogaster tennesseensis*. She runs about in the light chamber for several minutes, goes into the passage-way several times but not into the dark chamber. The *fulva* workers evidently detect her by her odor and one or two enter the light chamber and dart at her with abdomen turned under as though to grab her, but simply feel of her with their antennæ and allow her to run away.

9.45 A.M. Still in the light chamber.

4.45 P.M. I was compelled to be away during the day. The queen is now resting on the brood and is not being molested by the workers. One worker is licking her, although at times it seems to nab her.

5.30 P.M. The queen is licking the *fulva* larvæ. She moves about among the workers, none of which now molest her in the least. The pieces of the other two queens have been distributed among the larvæ and are being eaten by them.

July 27—8.00 P.M. The queen is resting on the larvæ with the workers and appears to be perfectly at home.

July 28—7.45 A.M. The queen is resting contentedly with the workers and larvæ. I was compelled to leave the experiment for a few days but during my absence one of the students in the laboratory recorded that on July 28—3.00 P.M. hostilities were again begun against the queen and that she was killed by 9.00 A.M. July 31.

Between August 2 and August 26 I placed in the nest in succession seven more queens of *Aphænogaster tennesseensis*, all of which were killed after a longer or shorter time.

The other colony of *Aphænogaster picea* which I used contained about forty workers, one queen, and a number of larvæ and pupæ. I placed the first queen in the nest on July 19 at 2.10 P.M. She was killed some time during the following night.

July 20—8.55 A.M. I place in another queen. She lives until 10.45 the next day. At 10.50 A.M. I place in a third queen and she succeeds in living until the morning of July 23. I also tried two queens of *Aphænogaster tennesseensis* with about twenty workers and some brood of the true *A. fulva*. Both queens were killed. The other experiments with colonies of *A. aquia* may be briefly summarized as follows:

B. 24*a*.

Contained ten workers, two winged queens, one male and brood. Between August 18 and September 1, I placed in three queens all of which were killed.

B. 24*b*.

Contained eight workers and a few pupæ. Between August 18 and September 7 two queens were killed.

B. 24*d*.

Contained eight workers and a number of pupæ. I placed in a queen August 29, which lived until September 4.

B. 24*e*.

Contained seven workers and a number of pupæ. I placed in a queen August 30 and it lived until September 4.

One other experiment with a queen of *Aphænogaster tennessensis* consisted in placing a dealated queen in a Petri dish with about thirty pupæ of *A. aquia*. The pupæ of this species, as with other species of myrmicine ants, are naked and do not require the assistance of workers in hatching. They were collected and placed in a Petri dish with the queen July 23. The queen paid no attention to them whatever. In a few days the first callows began to hatch and busied themselves taking care of the remaining pupæ. The queen at first paid no more attention to the callows than she had to the pupæ and in fact stayed in another part of the nest most of the time. After about a half a dozen had hatched, however, she began to stay with them more of the time. The callows were not hostile to her but readily adopted her as their queen. This, however, is what was to be expected because of the discovery made by Miss Fielde and recorded in her paper "Artificial Mixed Nests of Ants." She says: "If one or more individuals of each species that is to be represented in the future mixed nest be sequestered within twelve hours after hatching and each ant so sequestered touch all the others with its antennæ during the ensuing days, these ants will live amicably together thereafter although they be of different colonies, varieties, species, genera or subfamilies." At the present time, September 10, all the pupæ have hatched and have become adult workers, about thirty in all, and are clustered about the *tennesseensis* queen exactly as though she were their own.

Formica obscuriventris.

This subspecies of *rufa* occurs throughout the northern states east of the Mississippi River. It is fairly common in eastern Massachusetts and has been taken in Illinois. The queens are large, about the size of those of *F. sanguinea* var. *rubicunda*, and have shiny, jet-black abdomens with very little, almost no pubescence. The thorax and head are a darker red than in *F. sanguinea rubicunda*. The workers vary greatly in size.

There are no published accounts of the occurrence of this species in mixed nests in this country, but Professor Wheeler found such a nest this last spring near Boston, and has kindly given me permission to use the note which he made of it at the time. "April 10, 1910. On the northern slope of Great Blue Hill, under a stone, I found a mixed colony consisting of a dealated queen and about 80 workers of *Formica obscuriventris* with about 100 workers of *F. subsericea*. There were a few larvæ evidently of the former species in the nest."

On a trip to that same locality with Professor Wheeler, August 17 we came across a very large colony of this species which extended under several good sized stones from which we secured 10 dealated or partially dealated queens. The fact that they had been partially dealated indicates that they had probably been fertilized and readopted by the colony. The particular slope upon which this nest was found was very rich in nests of *F. subsericea*. I brought the queens of *F. obscuriventris* to the laboratory and also a large number of workers and brood from a colony of *F. subsericea*.

I divided the *subsericea* colony into five groups of workers and brood and tried one or more of the *obscuriventris* queens with each group. The behavior on the part of the queen and workers was the same in each case. Some of the workers attacked the queen, some began at once to lick her and others alternated between licking and biting her. The queen always seemed to show surprise at receiving such harsh treatment so different from what she was accustomed to receiving from the workers in her own nest. Then she would try to get away and finding that impossible would turn and bite a worker, sometimes producing a wound that caused death. If the *subserica* colony was a large one the queen was soon killed, but in the smaller colonies the workers, after a time, ceased their attacks and began to tolerate her presence in the nest, while more and more of them began to treat her as their own queen. I will give full notes on one experiment which shows the difference in results attained in using large and small colonies.

B. 22*b*.

August 18—11.30 A.M. I place a queen (I think the mother queen from the colony) of *Formica obscuriventris* in light chamber of a Fielde nest containing

about 75 workers, one male and many pupae of *F. subsericea*. These *subsericea* are all very large individuals.

The workers attack her fiercely when she is in the passageway. Some pull her toward the dark chamber while some pull the other way. They finally get her into the dark chamber where six or seven attack her at once.

12.00 M. She is in the dark chamber, workers still attacking her.

1.30 P.M. She is on the sponge in the dark chamber and is motionless. One worker is holding her by a middle leg, three others are licking her.

1.35 P.M. The one worker has let go her hold and four others are licking her. She seems to be dead.

2.30 P.M. The queen is dead. I change the light so as to make the other chamber the dark one.

4.00 P.M. They have moved the dead queen and the pupæ into the other chamber. I change the light again.

4.30 P.M. They again move the dead queen. I change the light again and when about two thirds of the workers are in the other chamber I block the passageway, leaving about two dozen workers, the dead queen and most of the pupæ on one side. I then place in another *obscuriventris* queen with these. They attack her also.

4.45 P.M. Two workers are holding her by the legs and two are licking her.

6.00 P.M. Four workers are holding her by the legs.

August 19—8.20 A.M. Two workers holding her, one by a leg and one by an antenna.

11.45 A.M. Two workers holding her.

2.15 P.M. The two workers still holding her.

3.00 P.M. The queen is dead. I remove the plug and let all but twelve of the workers escape into the other chamber and then replace the plug.

3.50 P.M. I place in another queen with the twelve workers; they attack her. She does not attempt to bite them except when a worker gives her an unusually painful jerk or bite.

4.00 P.M. One worker is licking her and one holding her.

August 20—8.00 A.M. One worker holding her by the leg.

2.00 P.M. Three workers standing near her; none holding her.

6.00 P.M. Two workers holding her.

August 21—11.00 A.M. The queen is standing alone.

August 22—11.00 A.M. She is standing with three workers; they do not attack her.

5.30 P.M. Two workers holding her.

August 23—9.00 A.M. Not attacking her this morning; two workers staying with her.

2.00 P.M. Still standing with two of the workers.

Sept. 10. I have examined this nest several times every day since August 23 and have never seen the queen attacked. There are now ten workers and a number of larvæ and pupæ in the nest, and the queen stays with them all the time exactly as though she were their own.

The other experiments with queens of *F. obscuriventris* may be summarized as follows:

B. *22a.*

August 18. I placed an *obscuriventris* queen in a Petri dish with eight workers and some pupæ of *F. subsericea*. The workers at first attacked her and continued doing so at intervals until noon the next day. After that they did not attack her, but tolerated her presence in the nest and she remained standing by herself until noon August 23. After that she stayed with the workers all the time and was treated as their own queen.

B. *22c.*

August 19. I placed an *obscuriventris* queen in a Petri dish with eight workers and twelve pupæ of *F. subsericea*. She was attacked at first just as the others were and the workers remained hostile until about the middle of the following day when they became indifferent to her presence and by August 25 they adopted her and treated her as their own queen. She had received an injury however in the first attacks from which she did not recover and by the morning of August 27 was dead. There were then six workers in the nest and one of them seemed weak. I removed the dead queen and placed in another. I did not see her attacked after the first day and by a day or two later she was fully adopted by the five workers, the other one having died.

B. *22d.*

August 24. I placed an *obscuriventris* queen in a nest containing about fifty workers, one male and many naked pupæ of *F. subsericea*. She was vigorously attacked, but within five minutes after she was placed in, while four workers were holding her, two others were diligently licking her thorax and abdomen. There were too many hostile workers in the nest however and by the following morning she had been killed and beheaded.

B. *22e.*

August 29—4.00 P.M. I placed an *obscuriventris* queen in a Petri dish with ten workers and eight pupæ of *F. subsericea*. She was attacked by the workers that afternoon, but on the following morning was standing by herself on the opposite side of the dish from where the *subsericea* were with their pupæ, and remained thus for two days. On the third day I disturbed the nest a little, and she moved over and mingled with the workers. They did not attack her and from then on, she stayed with them most of the time and is now, September 10, fully adopted.

It will be seen in the above experiments that of the 8 queens of *F. obscuriventris* tried with workers of *F. subsericea*, 5 were adopted, that these 5 were with colonies of from 8 to 12 workers and that those that were not adopted were with colonies of from 25 to 75 workers. The ease with which these queens are adopted in small colonies of *subsericea*, the decided tendency on the part of many of the workers, even in the large colonies, to begin licking and caressing the queens almost from the first, the inquilinous tendency shown in the behavior of the queens themselves and especially the finding of the mixed colony mentioned in the note

above, give conclusive evidence of the fact that the queens of *F. obscuriventris* are, at least in part, temporary parasites on *F. subsericea*, and the fact that a number of partly dealated and therefore probably fertilized queens had been retained in the large colony may be taken as an indication that, once formed, the colony may grow by budding as is the case with the large mound ant, *F. exsectoides*, which has also been shown (Wheeler, 1906) to be temporarily parasitic upon *F. subsericea*.

Lasius (Acanthomyops) latipes.

This species not only possesses aberrant females but is peculiar in the fact that it has two distinct forms of females as shown in a paper by Wheeler and McClendon, "Dimorphic Queens in an American Ant." These two queens have been designated by them as alpha and beta, the beta being the more aberrant but the more common, and up to the time of the appearance of the above mentioned paper, the only one known. The alpha queen, as shown by measurements given in that paper, is almost exactly intermediate between the beta female and the normal female of *L. claviger*. The two more probable of the four possible hypotheses suggested therein in explanation of the occurrence of these two forms are

1. That the dimorphism may be regarded as the result of hybridism between *L. claviger* and *L. latipes*.

2. That it may be a case of true dimorphism in the female sex.

In a recent paper, "An Aberrant Lasius from Japan"[1] Wheeler, after stating that he had made many observations in the field which showed that the queens of the following species of *Lasius*, *L. americanus*, *L. neoniger*, *L. nearcticus*, *L. brevicornis*, *L.* (*Acanthomyops*) *claviger* and *L.* (*Acanthomyops*) *interjectus* are able to establish their colonies independently, adds: "But I have never seen any of the females of our *umbratus* forms (*mixtus* var. *aphidicola* Walsh, *subumbratus* Viereck, *minutus* Emery and *speculiventris* Emery) in the act of founding their colonies independently, and it is quite probable that they are temporary parasites on the extremely common *L. americanus*. Equally negative have been my observations upon *L.* (*A.*) *latpies* which

[1] Biological Bulletin, 1910.

has the alpha and beta females. . . . That this species is a temporary parasite on *L. americanus* is indicated by the fact that near Colebrook, Conn., I found four small mixed colonies." The four mixed colonies mentioned here are the only ones of which there is any published account, but Professor Wheeler found another such colony this last spring and has given me the following note which he made at the time.

"Ellisville, Mass., April 21, 1910 Found a large mixed colony of *L. americanus* and *Acanthomyops latipes*, both about equally numerous and the *latipes* were no larger than the *americanus*. The nest was under a stone and contained a number of larvæ so young that I could not tell to which species they belonged. Both species took part in carrying the larvæ to a place of safety when the stone was raised, but the *latipes* were much more active in this pursuit than the *americanus*. The members of the two colonies were on the most friendly terms and occupied the same burrows."

The above facts furnished very good reasons for trying *latipes* queens with other species of *Lasius* and especially with *L. americanus*. On a field trip with Professor Wheeler in the Litchfield Hills near Colebrook, Conn., we came across a very large colony of *L. latipes* under a stone from which I obtained more than 75 winged females, all but 3 of which were beta females. These, and a number collected about two weeks later by Professor Wheeler, part of them from a colony in the same locality and part of them found crawling over the ground near Canton, Conn., after they had descended from their nuptial flight, were the ones used in the experiment.

Altogether I tried 79 queens of *L. latipes* with 28 different colonies of 8 different species of *Lasius* divided as follows: 14 colonies of *L. americanus*, 4 colonies of *L. nearcticus*, 4 colonies of *L. claviger*, 1 colony of *L. claviger* var. *subglaber*, 1 colony of *L. brevicornis*, 2 colonies of *L. interjectus*, 1 colony of *L. umbratus* var. *minutus* and 1 other colony of *L. latipes*. Out of all these I got but two clear cases of adoption in which the queen lived, one of these being an alpha, the other a beta female. However, not nearly all the deaths among the queens experimented with were due to the hostilities of the other ants, for the queens of

this species do not keep well in confinement and during the time the experiment was running more than 100 females not used in experiments died, part of them in the colony with their own workers and part of them in a nest by themselves. I am quite sure that at least four or five and probably more of the queens with *L. americanus* were already adopted or would have been adopted had they not died. In the majority of cases when I removed the dead queen I was unable to find any trace of injury whatever although in a number of cases the body was dismembered and sometimes eaten.

The most noticeable feature about the behavior of the *latipes* female when placed in with a small colony of workers was her desire to be with the brood, although in only one case out of all 79 did I see a queen pick up a larva or cocoon. This queen was in a Petri dish with a few workers of *L. nearcticus* and once when I uncovered the dish she picked up a cocoon and carried it around in her mandibles for about a minute. The queen was always attacked, and in the larger colonies of *americanus* and in the one of *L. umbratus minutus*, very fiercely. I did not see her in any case attempt to defend herself. In the larger colonies she only tried to get away and in the smaller colonies she usually got on the pile of cocoons, while the little workers attacked her and crawled over her large body in their efforts to kill her. Time and again when they succeeded in dragging her away she would return and mount the pile of cocoons. The callows hatching under these conditions would not, of course, be hostile to her and as at this time of the year the callows were emerging in large numbers there would soon be many workers about her that would accept her as their own queen. Even in the larger colonies a number of workers could often be seen licking the queen while others were attacking her. I will give a few notes on the colonies in which the adopted queens lived, and summarize the others.

B. 19/8 August 9. I place a beta queen of *L. latipes* in Petri dish with twelve workers of *L. americanus* and 150 cocoons.

Aug. 10. The queen is dead.

Aug. 11. I place in another.

Aug. 12. She is on the pile of cocoons with a large number of newly hatched workers.

Aug. 13. Still resting on the cocoons with the callows.

Aug. 14. The queen and callows are clustered together on the brood.

Aug. 15. The queen is almost hidden from sight by the workers that are clustered about her and over her. They are all resting on the pile of cocoons.

Aug. 16. The same.

Aug. 17. The same. Many of the workers licking her.

Aug. 18. She is entirely hidden by the workers clustered over her. I have examined this nest every day up to the present time, September 12, and the workers have always been clustered about her. This is much more pronounced than is the case even with the rightful queen in a colony of *americanus*. There are now more than 100 workers in the nest.

B. 19/13 Aug. 10. I place a beta queen of *L. latipes* in Petri dish with 30 workers and about the same number of cocoons of *L. interjectus*.

Aug. 11. Dead. I place in another alpha queen.

Aug. 12. She is staying with the workers on the pile of cocoons and seems to be perfectly at home.

Aug. 13—9.00 A.M. She is near the pile of cocoons with a bunch of workers. I watch them for 30 minutes this morning and do not see the workers show the slightest sign of hostility although they are touching her and climbing over her all the time.

6.00 P.M. The workers are licking the queen with every evidence of satisfaction.

I have examined this colony every day up to the present time, September 12, and the behavior on the part of both the queen and the workers has always been the same.

In the following experiments not more than one *L. latipes* queen was in a colony at the same time, and although in the summary I make the statement that the queens were killed it should be remembered that probably the majority of them would have died even if they had been in artificial nests with their own workers since more than 100 died that were not used in the experiment.

B. 9.

Colony of 150 workers and brood of *L. americanus;* 1 queen killed.

B. 16.

60 workers and brood of *L. americanus;* 3 queens killed. The last one stayed in the nest 17 days; I think she had been adopted.

B. 17*b*.

300 workers and much brood of *L. claviger;* 1 queen killed.

B. 17*c*.

25 workers, 3 winged females, 5 males, and cocoons of *L. claviger* var. *subglaber*. 3 queens killed, one of these an alpha queen.

B. 19/1. 20 workers of *L. claviger* and brood; 3 queens killed.

B. 19/2. 20 workers and a few cocoons of *L. americanus;* 3 queens killed.

B. 19/3. 20 workers and a few cocoons of *L. americanus;* 5 queens killed.

B. 19/4. 40 workers and 100 cocoons of *L. americanus;* 7 queens killed.

B. 19/5. 20 workers of *L. americanus* and 200 cocoons; 6 queens killed.

B. 19/6. 20 workers and 200 cocoons of *L. americanus;* 6 queens killed.
B. 19/7. 20 workers and 150 cocoons of *L. americanus;* 6 queens killed.
B. 19/8. 12 workers and 100 cocoons of *L. americanus;* 3 queens killed, 1 an alpha.
B. 19/10. 30 workers, 7 queens, no brood of *L. nearticus;* 6 queens killed.
B. 19/11. About a dozen workers, 30 cocoons, of *L. nearticus;* 2 queens killed.
B. 19/12. About a dozen workers, 1 male and 30 cocoons of *L. nearticus;* 1 queen killed.
B. 19/14. 30 workers, no brood, of *L. claviger;* two queens killed.
B. 19/15. 50 workers, many cocoons of *L. nearticus;* 1 killed.
B. 19/16. 36 workers, many cocoons of *L. brevicornis.* 2 queens killed.
B. 19/17. 8 workers and many cocoons of *L. americanus;* 4 queens killed.
B. 19/18. 4 workers and many cocoons of *L. americanus;* 3 queens killed.
B. 19/19. 6 workers and a few larvæ of *L. americanus;* 2 queens killed.
B. 19/20. 30 workers and many cocoons of *L. americanus;* 2 queens killed.
B. 19/21. 30 workers and no brood of *L. minutus;* 1 queen killed.
B. 19/22. 12 workers and no brood of *L. interjectus;* 1 queen killed.
B. 19/23. 25 workers and brood of *L. latipes;* 1 queen killed.

The fact that more queens died in some of the colonies than others has no significance, for in some of them I did not replace the first queen.

We thus have two positive cases of adoption of queens of *L. latipes* by workers of other species of *Lasius* and the five records given above show that *latipes* occurs in mixed colonies in nature. At first thought it might seem that 2 adoptions out of 79 attempts is entirely too small a percentage upon which to base any conclusions whatever regarding the point of temporary parasitism. Yet considering the fact that nests of *L. latipes* are not found abundantly in nature and that the queens are produced in the colonies in large numbers would we expect a larger number of adoptions? It is very doubtful whether even in *L. americanus* 2.5 per cent. of the queens succeed in founding colonies.

Another possible explanation for the mixed colonies, however may be suggested by an observation which I made on September 5. There had been a nuptial flight of *L. americanus* and *L. latipes* and as I was walking through a park reservation not far from the Bussey Institution between five and six o'clock in the evening, I picked up off the ground 30 dealated females of *L. americanus* and 5 dealated beta females of *L. latipes.* As I had but one box with me I placed the queens of both species together and upon returning to the laboratory a few minutes later, dumped them all together in the same nest. After a few

minutes the *americanus* queens were huddled together near a moist sponge I had provided for them but the *latipes* queens, always more restless in confinement, were still running about in the nest. They were continually running over or through the bunch of *americanus* queens and sometimes remained with them for several minutes, yet I never saw the slightest signs of hostility either on the part of the *americanus* or the *latipes* queens. The next morning 3 of the *latipes* queens were dead and two days later the other 2 died, yet I feel quite sure that death was not caused by any hostility on the part of the *americanus* queens. In the same length of time 7 *americanus* queens died, but there have been no more deaths up to the present time. I feel sure that a number of the deaths of the *americanus* queens were due to injuries received when I picked them up off the ground. The fact that the nuptial flight of *latipes* and *americanus* may occur at the same time and that the queens of the two species are not hostile to one another suggests the possibility of a colony being founded in common by queens of the two species. This possibility should be tested by experiment. However, I think temporary parasitism a more plausible explanation of the mixed colonies mentioned above because of the fact that the *latipes* queen is of a more nervous temperment, and even though there were no hostilities between the two queens she would not be satisfied to settle down in a little cell with the phlegmatic *americanus* queen and wait nine or ten months for the appearance of workers. This nervous disposition, however, is exactly suited to running about over the ground until the queen happens to run into a small Lasius colony, and when she gets on to the brood she is perfectly satisfied to settle down as is shown by the experiments.

The adoption of the *latipes* queen by the colony of *L. interjectus* may be looked upon as adding weight to one of the explanations quoted above for the occurrence of the two forms of females, namely, that the alpha form may be a hybrid between the beta female and a male *claviger*. Since adoption occurred with *interjectus* it might also be expected to occur with the nearly related *claviger* if enough cases were tried. Again the females of *claviger* and *interjectus* are very similar so that the alpha form

might just as well be a hybrid between *latipes* and *interjectus* as between *latipes* and *claviger*.

Lasius umbratus var. minutus.

One of the quotations given above shows the lack of evidence that the queens of this species are able to found their colonies independently. The lack of such evidence taken together with the fact that the ant is sporadic in its occurrence, that it produces an immense number of the sexual forms and that the females differ from all the other *Lasius* females in being very small, no larger than the largest workers, point to temporary parasitism as a method of colony formation.

On August 12 I came across a large mound nest of this species at the edge of a forest reservation near the Arnold Arboretum. The mound was in the shape of a very broad dome, about eighteen inches high and about three feet across at the base. Judging from the size of the nest, the number of individuals and the way the grass was shot up through the mound the colony must have been several years old. With a trowel I took out about a quart of dirt from the side of the mound and brought it to the laboratory. I found that I had about 150 females, an equal number of males, several hundred workers and many cocoons. As the amount of earth I removed hardly made an impression on the mound the colony must have contained several thousand males and females and a still larger number of workers. Although I collected rather extensively in that region and in a number of other localities around Forest Hills, this is the only colony of *minutus* that I found during the entire summer. In my experiments with this species I used 88 queens in 20 different colonies as follows.

B. 25*a*.

20 workers, 2 winged queens and brood of *L. americanus*.

B. 25*b*.

8 workers and many pupæ of *L. americanus*.

B. 25*c*.

8 workers and many pupae of *L. americanus*.

B. 25*d*.

30 workers and many pupæ of *L. americanus*.

B. 28/1. 7 workers, no brood of *L. claviger*.

B. 28/2. 24 workers and a few cocoons of *L. americanus*.

B. 28/3. 12 workers, and brood of *L. americanus.*
B. 28/4. 6 workers and a few larvæ and pupæ of *L. americanus.*
B. 28/5. 100 workers, and cocoons of *L. americanus.*
B. 28/6. 100 workers, and cocoons of *L. americanus.*
B. 28/7. 75 workers, 1 winged queen and many ocoons of *L. americanus.*
B. 28/8. 12 workers, no brood of *L. interjectus.*
B. 28/9. 25 workers, and brood of *L. americanus.*
B. 28/10. 25 workers and 5 winged queens of *L. nearticus.*
B. 28/11. 24 workers, and cocoons of *L. americanus.*
B. 28/12. 100 workers and many cocoons and young larvæ of *L. americanus.*
B. 28/13. 12 workers, and cocoons of *L. americanus.*
B. 28/14. 24 workers, and cocoons of *L. brevicornis.*
B. 28/15. 50 workers, and cocoons of *L. nearticus.*
B. 28/16. 30 workers and a number of cocoons of *L. americanus.*

The queen of this species is very active and very timid. I did not see her attempt to defend herself in any case but always tried to escape and by her active movements she was usually able to get away from the workers for quite a while. When caught, however, she usually succumbed in a much shorter time than was the case with the other species with which I worked. A few of them died within an hour or so after being placed in, others living for several days. Out of all these experiments I got but one case of adoption. I will give a few notes from that experiment.

B. *25b.*

Aug. 18—3.30 P.M. I place a queen of *L. umbratus* var. *minutus* in a Petri dish with 8 workers and a large number of pupæ of *L. americanus.* The workers attack her; she does not defend herself but tries to escape from them.

Aug. 19—9.05 A.M. She is running around by herself. A worker attacks her for a little while but she gets away.

Aug. 20—9.30 A.M. She is resting on the cocoons with the workers.

Aug. 21—9.00 A.M. The same.

11.00 A.M. Still on the cocoons with the workers and seems to be entirely immune from attack. About a dozen callows have hatched.

Aug. 22. The same.

Aug. 23. The same.

Aug. 24. There are about 40 or 50 workers in the nest now. The queen stays with them all the time.

Sept. 12. Most of the cocoons have hatched. The queen has been fully adopted.

Although one adoption out of 88 attempts is a small percentage, yet I think the ease with which this queen was adopted is very suggestive, and taken altogether with the facts mentioned above, namely the sporadic occurrence of the species, the very large number of females produced, the small size of the females,

the fact that these females have not been seen in the act of founding a colony and one additional fact which may be mentioned, the mimetic coloration of the females (the color of these females is exactly the same as that of the darker form of *americanus*), I think justifies us in concluding that the queen of this species is in all probability, temporarily parasitic upon the common *L. americanus*.

Polyergus lucidus.

This shining slave-maker has been studied by Mrs. Treat, McCook, Burill, and Wheeler,[1] and the European form *P. rufescens*, by Huber, Forel, Wasmann and Viehmeyer. It differs from the ants mentioned above in that it is not a temporary but a permanent parasite or slave-maker, the workers making raids upon colonies of *Formica schaufussi* and its varieties *incerta* and *nitidiventris*.

Studies by Forel, Wasmann and Viehmeyer on the European *P. rufescens* tend to show that that form resembles the temporary parasites in that the queens may be adopted by fusca workers. In regard to the founding of colonies by *P. lucidus* Wheeler says:[2] "Several experiments in which I introduced artificially dealated queens of *lucidus* into nests containing *incerta* workers with their brood gave rather conflicting results. In some cases the *lucidus* queens behaved like the *sanguinea* queens under similar conditions to the extent of killing the alien workers, but they paid absolutely no attention to the brood. In other cases they were passive and conciliatory but equally indifferent to the *incerta* cocoons. It will be necessary therefore to study this question further before making definite statements in regard to the methods employed by our American amazons in establishing colonies."

The queens used in the following experiments were obtained on the slope of Blue Hill, August 17, the same date upon which we found the large colony of *F. obscuriventris* mentioned above. The nest was along the side of a little-used roadway. On excavating and bringing it to the laboratory I found the colony to contain about a dozen workers, an equal number of winged

[1] See Wheeler, "Ants, Their Structure, Development and Behavior," pp. 482–487.

[2] "Ants, Their Structure, Development and Behavior," p. 486.

queens, one dealated queen of *P. lucidus*, and about 100 very small workers of *F. incerta* and a number of cocoons. The small number of *lucidus* workers and the comparatively large number of females indicates that many of the workers were probably out on a slavemaking raid at the time.

I tried seven queens with colonies of *F. incerta* and four with *F. schaufussi*. Two out of the seven were clear cases of adoption in which the queens are still living. One of the others I think was without doubt adopted but died later on from injuries received in the first struggles. The two *schaufussi* colonies consisted of very large individuals and each contained a queen. They attacked the *lucidus* queens more fiercely than did the *incertas*, and all four were finally killed, yet in both colonies I saw at times a few of the workers licking the queens and I think it would be possible if enough cases were tried, to secure adoption with *F. schaufussi*, especially if the colonies did not contain queens of their own.

The behavior of the *lucidus* queens was like that of the *obscuriventris* queens in that they did not at first attack the workers but ran about in the nest avoiding them and trying to escape. However, when caught and held by the workers they were not nearly so submissive as were the *obscuriventris* queens and when forced to defend themselves they did so with such vigor and persistence, pierceing the head or thorax of the unfortunate workers with their long sickle-shaped mandibles so that a very large number of the *incertas* or *schaufussi* workers were killed. In one of the *schaufussi* colonies the *lucidus* queen killed every one of the fifteen workers, leaving only the *schaufussi* queen. Later on, however, the *lucidus* queen herself died. My results agree with those of Wheeler mentioned above in that I did not see a queen at any time pay the slightest attention to the brood, her behavior being different in this respect from *F. sanguinea*, which, as Wheeler has shown, kills off the workers, takes possession of the brood and frees the first callows from their pupal envelopes. I will give a few notes from the two experiments in which the queens were adopted and are still living.

B. 23*a*.

Aug. 18—2.00 P.M. I place a dealated queen of *P. lucidus* in Petri dish with two dozen workers and 8 pupæ of *F. incerta*. They attack her at once. She tries to escape from them and usually manages to do so as she is very vigorous. She does not attack them, and bites them only when she cannot get away.

2.20 P.M. 3 workers holding her, one by a leg and one by an antenna.

4.00 P.M. Still being held by 2 workers.

5.00 P.M. The same.

6.00 P.M. The same.

Aug. 19—8.20 A.M. 3 workers holding her on the sponge. This hostility was continued against the queen until the morning of Aug. 23 when I found her dead.

Aug. 23—1.45 P.M. I place in another *Polyergus* queen. All but one of the cocoons have hatched. Some of the workers escaped, but two have been killed. There are now 15 workers in the nest and 1 of them is injured. The injured one attacks the queen and is soon killed by her. None of the other workers attempt to attack the queen. She runs about in the nest and attempts to escape.

4.00 P.M. 3 workers holding her, 2 by the antennæ and 1 by a leg.

4.30 P.M. 3 workers are licking her.

6.00 P.M. 1 worker is holding her by a leg.

Aug. 24—8.00 A.M. 2 workers are standing by her side; they do not attack her and she does not avoid them.

3.30. 1 of the workers is licking her.

4.00 P.M. 3 workers are licking her. A few minutes later one of them holds her by the tarsus for a while.

Aug. 25—8.00 A.M. The *Polyergus* queen is dead.

2.45 P.M. I place in another queen.

Aug. 26—9.00 A.M. She is standing in the midst of the workers and seems to be entirely uninjured. The workers do not attack her. There are several dead workers in the nest.

11.45 A.M. She moves around with the workers and does not try to avoid them, nor do they avoid her nor show any hostility to her.

Aug. 27—8.00 A.M. The workers are standing all around her; there are 10 left alive.

Sept. 12. I have examined the nest several times every day up to the present time and have never seen her attacked. She has been fully adopted.

B. 23*b*.

Aug. 19—11.20 A.M. I place a *Polyergus* queen in one chamber of a nest containing the mother queen, 16 workers and a few larvæ and pupæ taken from a large colony of *F. incerta*. The *Polyergus* queen runs about in the nest trying to escape. The workers are afraid of her and only a few try to attack her. They gather up their pupæ as they would in case of a raid.

1.45 P.M. All but 6 of the workers escape past the cotton plug into the other chamber.

6.00 P.M. The *Polyergus* queen is standing by herself in a corner of the nest The *incerta* queen and workers are standing in another corner with the brood.

Aug. 20—8.00 A.M. The same.
2.00 P.M. The same.
Aug. 21—10.30 A.M. The same. The *Polyergus* queen kept aloof from the *incertas* all the time until August 24. When I examined the nest at 1.00 o'clock on that day the *Polyergus* queen was running around in the nest but avoiding the *incertas* and they seemed afraid to attack her. About an hour later, however, I found the *incerta* queen dead and the *Polyergus* queen standing near her. I removed the dead queen and examined her. I found four cases where her thorax had been punctured by the sharp mandibles of the *Polyergus* queen. From that time on the *incerta* workers not only did not attack the *Polyergus* queen but they ceased to avoid her, and and on the following day I found one of the workers licking her. Since then they have treated her as they treated their own queen before. There are 8 workers in the nest now.

Besides the experiments with the above mentioned ants I tried a few adoption experiments with queens of *F. nepticula*, *F. sanguinea* var. *rubicunda*, and one with a queen of *F. difficilis* var. *consocians*.

The queens of *F. nepticula* were tried with small colonies of *F. inserta*, *F. fusca* var. *subænescens* and *F. subpolita* var. *neogagates*. The two queens that I tried with *incerta* and *subænescens* gave negative results. The workers attacked the queens fiercely in each case. The active little queen defended herself, however, by seizing them with her mandibles. The movements made the first few minutes were so rapid that the eye could scarcely follow them. In an hour or so, however, the queen was killed in each nest. The behavior was the same when I placed a queen in with a small colony of *F. subpolita*, but the *subpolita* workers being smaller than those of the other two species were not able to kill the queen so quickly, and after a fierce struggle of a few minutes she escaped from them. Although she was attacked again from time to time the attacks were not so fierce and several times I saw workers licking her; not only that, but her behavior then became more like what Wheeler has described for the queens of *F. consocians* when introduced into a colony of *F. incerta*, more insinuating and conciliatory, and twice I found the *nepticula* queen feeding a *subpolita* worker. Although the two queens which I tried with this *subpolita* colony were both finally killed I think the behavior both on the part of the queen and the workers tends to confirm the conclusions reached by Wheeler in 1906, that *F. nepticula* is a temporary parasite and that its probable host is *F. subpolita*.

The four queens of *F. sanguinea* var. *rubicunda* which I tried with small colonies of *F. subsericea* were not in very good condition as I had kept them too long in confinement without food and with one exception were killed. This one, however, behaved as did those in the experiments described by Wheeler in 1906. She killed four of the eight workers in the nest and after some time took charge of the pupæ. The other four workers remained hostile for about two weeks, after which they adopted her and helped take care of the brood.

The one colony of *incerta* with which I tried a queen of *F. consocians* contained the mother queen, about three dozen workers and several cocoons. I placed the *consocians* queen in with them on August 5 at 4.30 P.M. The introduction of the queen caused very little disturbance. The workers she met nabbed at her and pulled her legs and antennæ a little but not at all violently. Within an hour after being placed in she was fully adopted and was going about feeding the workers by regurgitation. The two queens, *incerta* and *consocians*, lived peacefully side by side. I still have the colony in the laboratory, September 16, and the yellow *consocians* queen seems to be perfectly happy with her strange nest mates. While on a field trip with Professor Wheeler near Colebrook, Conn., July 30 and 31, he found two mixed colonies of *incerta* and *consocians* and has given me the following notes which he made of them:

"Colebrook, Conn., July 30, 1910.

"No. 1. A mixed colony (in the second year) consisting of a *consocians* female and about 100 workers with brood, and a somewhat smaller number of workers of *incerta*. This was under a stone on Mt. Pisgah.

"No. 2. Colebrook, Conn., July 31, 1910. At Beech Hill near the Massachusetts boundary north of Colebrook I found a large and flourishing colony of *F. incerta* containing fully 200 workers and much brood, containing a single *consocians* queen that must have been very recently adopted. This is the largest *inserta colony* in which I have found a *consocians* queen."

The finding of mixed colonies of these two species, *incerta* and *consocians*, near Colebrook, Conn., a number of years ago and subsequent extensive field observations and experiments

is what led to the discovery by Wheeler of the phenomenon of temporary parasitism among ants. At the same time he also showed that temporary parasitism exists in other American ants and predicted that it would be found to exist in certain European ants. These predictions have since been verified by a number of European myrmecologists. The results of the experiments recorded in this paper verify his predictions concerning the queens of *A. tennesseensis*, *L. latipes*, *L. umbratus minutus* and show that *F. obscuriventris* is a temporary parasite upon *F. subsericea* just as he has shown that the European *F. rufa* is parasitic upon the typical *F. fusca*.[1]

A question which will naturally arise in the minds of most people is, "To what extent would it be possible to secure the adoption of non-parasitic queens by workers of different species or even of different colonies of the same species?" Much light would be thrown on the whole subject if an extensive series of experiments with such queens should be conducted, and in the future I hope to be able to perform such experiments. Also, the logician will say that it should not only be proved that queens that are supposed to be temporary parasites may be adopted by workers of another species but it should also be proved that such queens are incapable of founding colonies unaided. That such is the case with some of the queens experimented upon we have only such negative evidence as I have given above, and positive evidence, one way or the other, can be obtained only by an extensive series of careful experiments. However, positive evidence that any of the queens given above as temporary parasites are able to establish colonies independently would not necessarily prove that they may not also be in part temporarily parasitic upon some other species. It is more than likely that a very large number, perhaps most of the ants are in some stage of development toward parasitism. For instance, the above experiments show that the queens of *F. obscuriventris* are not so easily adopted as those of *F. consocians* but much more easily than those of *F. neoticula* or of *A. tennesseensis*. Wheeler has shown that the first step toward both temporary and permanent parasitism from the primitive independent type of colony for-

[1] "Observations on some European Ants," 1909.

mation is the facultative adoption of the queen by workers of the same species, the second the obligatory adoption of the queen by workers of the same species, and third the obligatory adoption of the queen by workers of another species. Perhaps the last species in which one would look for traces of even the first steps toward parasitism would be our dominant species *L. niger* var. *americanus*, since it is well known that the queens of this species are able to extablish colonies independently. Workers from one colony of this species are always very hostile to those belonging to another colony and still more so towards queens from another colony, yet, out of eight attempts I succeeded in getting one young fertilized and dealated queen adopted by a colony of *L. americanus* consisting of about three dozen workers, six cocoons and a number of very small larvæ. It is therefore very likely that many of the queens of *americanus* establish their colonies with the aid of workers either of the same or of a different colony. Thus we see that even in our most dominant species we may find the first two steps toward parasitism. It is easily seen, therefore, that there is still a vast amount of work to be done before the last word can be said upon the interrelations of the different species of ants.

In conclusion I wish to thank Professor W. M. Wheeler, under whose direction the work was done, for his many helpful suggestions while the experiments were being conducted and for reviewing my manuscript.[1]

[1] The colonies containing the adopted queens were left at the Bussey Institution and Mr. J. W. Chapman, who was kind enough to care for them, wrote me on November 25, that in each case the adopted queen was treated just as though she were the rightful queen, so that there is no question but that the adoption was complete.

M. C. T.